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Influence of Agricultural Management Practices on the Restoration of Marginal Land with Special Emphasis on the Development of Plant-Microbe Interactions

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Abstract

The globally increasing demand for food and biomass of the growing world population together with rising land use activities and the effects of climate change are leading to a tremendous pressure on fertile soils and agricultural productivity. To ensure global food security under these conditions, agricultural management must be improved and ecosystem services must be protected. Facing these challenges, the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI), funded by the EU, raised the project INTENSE which aims to intensify agricultural production, transform biomass to energy and novel goods and to protect soils in Europe.

In this context, organic soil amendments, which are obtained after energy (e.g. biochar, digestate) or food production (e.g. spent mushroom substrate), have raised considerable interest since they may provide an array of positive effects on soil health and plant performance. Nevertheless, since also negative effects may arise after amendment application we investigated exemplarily the impact of pelletized spent mushroom substrate blended with digestate and straw, together with different combinations of biochar and mineral fertilizer, on soil quality and performance of barley plants (*Hordeum vulgare* L.) in a greenhouse experiment. To further decrease the pressure on fertile arable soils, also the gentle restoration of previously neglected and marginal sites should be considered. Therefore, we transformed a marginal grassland to arable land and examined the effects of grassland removal, tillage, intercropping with faba bean (*Vicia faba* L.) and its later incorporation on soil nutrient stocks and soil quality.

The results revealed strong effects on soil nutrient stocks and soil bacteria during grassland conversion and after fertilizer application in the barley experiment. Although a clear influence of agricultural management and plant development on soil bacteria became evident, the bacterial composition on phylum level remained quite stable during conversion and even under the influence of different fertilizers. However, on family and species levels strong changes of the bacterial composition were observed which were mainly depending on decomposition processes, plant development, nitrate levels, carbon/nitrogen ratio, and application of higher amounts of mineral fertilizer together with biochar. A slight decrease of bacterial diversity and evenness towards the final samplings indicated a shift to more dominant species, like *Massilia* which can stimulate plant growth, *Lysobacter* which support biological control of plant diseases, and *Mycobacteriaceae*

which balance excessive nitrate in soil. The unaffected microbial activity, enzyme activity, and metabolic diversity among the different fertilizer combinations in conjunction with changes observed on family level furthermore indicate strong functional redundancy. Both, the functional redundancy and the higher abundance of beneficial bacteria are good indicators for the resilience of the given soil and confirm the great potential of appropriate management practices (organic fertilization, gentle conversion) to restore marginal land. Moreover, the mineralization after breaking-up the grass scar in combination with the biological nitrogen fixation of *V. faba* and its later incorporation mobilized the nitrate pool in soil sufficiently for future cropping of arable plants. Besides, performance of barley plants in the greenhouse was improved by all fertilizer combinations, without revealing significant differences between organic amendments alone or with mineral fertilizer. This highlights the possibility of applying appropriate organic amendments, such as the pellets used, to lower or even replace the input of mineral fertilizer in environmentally sound farming. Noteworthy, the present holistic study revealed only beneficial and no detrimental effects of organic amendment application and highlights the importance of taking specific plant parameter (e.g. species, development stage, root exudates) into account when analyzing fertilization effects on soil bacteria.

Hence we conclude that organic amendments are suitable to intensify crop production, maintain soil health and to reduce the amount of wastes and residues in a more sustainable agriculture. Moreover, we find the intermediate cultivation of leguminous crops during grassland conversion appropriate to enrich soil nutrient stocks without harming soil quality and thus to unlock the potential of marginal land for future crop cultivation. However, we recommend long-term field studies to confirm our findings of suitable fertilizer combinations and gentle restoration strategies.

Zusammenfassung

Die weltweit steigende Nachfrage nach Nahrungsmitteln und Biomasse der wachsenden Weltbevölkerung zusammen mit zunehmenden Landnutzungsaktivitäten und den Auswirkungen des Klimawandels führen zu einem enormen Druck auf fruchtbare Böden und landwirtschaftliche Produktivität. Um die globale Ernährungssicherheit dennoch zu gewährleisten, müssen die landwirtschaftliche Bewirtschaftung verbessert und die Ökosystemleistungen geschützt werden. Angesichts dieser Herausforderungen hat die von der EU finanzierte Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI) das Projekt INTENSE ins Leben gerufen, welches die Intensivierung der landwirtschaftlichen Produktion, die Umwandlung von Biomasse in Energie und neuartige Güter sowie den Schutz der Böden in Europa zum Ziel hat.

In diesem Zusammenhang erlangen organische Bodenzusatzstoffe, die aus der Energie- (z.B. Biokohle, Gärreste) oder Nahrungsmittelproduktion (z.B. verbrauchtes Pilzsubstrat) gewonnen werden, zunehmendes Interesse, da sie eine Vielzahl positiver Effekte auf die Bodengesundheit und die Pflanzenleistung haben können. Da jedoch auch negative Effekte nach der Anwendung solcher Zusatzstoffe auftreten können, untersuchten wir exemplarisch die Auswirkungen von pelletiertem verbrauchtem Pilzsubstrat, das mit Gärrest und Stroh gemischt wurde, zusammen mit verschiedenen Kombinationen von Biokohle und Mineraldünger auf die Bodenqualität und die Leistung von Gerstenpflanzen (*Hordeum vulgare* L.) in einem Gewächshausversuch. Um den Druck auf fruchtbare Ackerböden weiter zu vermindern, sollte zudem die schonende Umwandlung bisher vernachlässigter Grenzertragsstandorte in Betracht gezogen werden. Deshalb wandelten wir unproduktives Grünland in Ackerfläche um und untersuchten die Auswirkungen von Grünlandumbruch, Bodenbearbeitung, Zwischenfruchtanbau mit Ackerbohne (*Vicia faba* L.) und deren späteren Einarbeitung auf die Nährstoffvorräte und Bodenqualität.

Die Ergebnisse zeigten einen starken Einfluss der Grünlandumwandlung und Düngung im Gerstenversuch auf die Nährstoffvorräte und Bakterien im Boden. Obwohl deutliche Auswirkungen der landwirtschaftlichen Bewirtschaftung und der Pflanzenentwicklung auf die Zusammensetzung der Bodenbakterien gezeigt wurden, blieb diese auf Stammebene während der Umwandlung und sogar unter Einfluss verschiedener Düngerkombinationen recht stabil. Auf Familien- und Artniveau jedoch wurden starke Veränderungen der bakteriellen Zusammensetzung beobachtet, die hauptsächlich von Zersetzungsprozessen, Pflanzenentwicklung, Nitratgehalt,

Kohlenstoff/Stickstoff-Verhältnis und der Ausbringung größerer Mengen an Mineraldünger zusammen mit Biokohle geprägt wurden. Eine leichte Abnahme der bakteriellen Diversität und Gleichmäßigkeit am Ende der Versuche deutet zudem auf eine Verschiebung zu dominanteren Arten hin, wie *Massilia*, die das Pflanzenwachstum stimulieren können, *Lysobacter*, welche die biologische Kontrolle von Pflanzenkrankheiten unterstützen, und *Mycobacteriaceae*, die übermäßiges Nitrat im Boden ausgleichen können. Die von verschiedenen Düngerkombinationen unbeeinflusste mikrobielle Aktivität, Enzymaktivität und metabolische Vielfalt in Verbindung mit den auf Familienebene beobachteten Veränderungen deuten zudem auf eine starke funktionelle Redundanz hin. Sowohl die funktionelle Redundanz als auch das höhere Vorkommen nützlicher Bakterien sind gute Indikatoren für die Widerstandsfähigkeit des gegebenen Bodens und bestätigen das große Potenzial geeigneter Bewirtschaftungspraktiken (organische Düngung, sanfte Umwandlung) zur Wiederherstellung von Grenzertragsböden. Außerdem mobilisierte die Mineralisierung nach dem Grünlandumbruch in Kombination mit der biologischen Stickstofffixierung durch *V. faba* und deren späterer Einarbeitung ausreichend Nitrat im Boden für den zukünftigen Anbau von Ackerpflanzen. Darüber hinaus wurde die Leistung der Gerstenpflanzen im Gewächshausversuch über alle Düngerkombinationen hinweg verbessert, ohne signifikante Unterschiede zwischen organischen Dünger allein oder mit Mineraldünger. Das verdeutlicht die Möglichkeit, mit geeigneten organischen Düngemitteln, wie z.B. den verwendeten Pellets, den Einsatz von Mineraldünger in einer umweltverträglichen Landwirtschaft zu verringern oder sogar zu ersetzen. Die vorliegende Studie zeigte zudem nur positive und keine nachteiligen Auswirkungen der Ausbringung organischer Zusatzstoffe auf und unterstreicht die Bedeutung der Berücksichtigung von spezifischen Pflanzenparametern (z.B. Art, Entwicklungsstadium, Wurzelexsudate) bei der Analyse der Auswirkungen von Dünger auf die Bakterien im Boden.

Hieraus kann man schließen, dass organische Düngemittel geeignet sind, die Pflanzenproduktion zu intensivieren, die Bodengesundheit zu erhalten und damit auch zu einer Reduktion von Abfällen und Reststoffen in einer nachhaltigeren Landwirtschaft beizutragen. Darüber hinaus halten wir den Zwischenanbau von Leguminosen während der Grünlandumwandlung für geeignet, die Bodennährstoffvorräte anzureichern, ohne die Bodenqualität zu beeinträchtigen, und damit das Potenzial von Grenzertragsböden freizusetzen. Langfristige Feldstudien sind jedoch notwendig, um unsere Erkenntnisse über geeignete Düngerkombinationen und schonende Restaurierungsstrategien zu bestätigen.

I. List of Abbreviations

ACC	1-aminocyclopropane-1-carboxylate acid
ANOVA	Analysis of variance
ASV	Amplicon sequence variant
bp	Base pairs
BC	Before Christ
BSA	Bovine serum albumin
C	Carbon
CG	Control grassland
Chl	Chlorophyll
DES	DNAse Free Water
DEPC	Diethyl dicarbonate
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
dw	Dry weight
EDTA	Ethylenediaminetetraacetic acid
EEA	Extracellular enzyme activity
FDA	Fluorescein diacetate assay (3',6'-diacetylfluorescein)
FS	Final state
fw	Fresh weight
FACCE-JPI	Joint Programming Initiative on Agriculture, Food Security and Climate Change
K	Potassium
IAA	Indole-3-acetic acid
IG	Initial grassland
INTENSE	Intensify production, transform biomass to energy and novel goods and protect soils in Europe
N	Nitrogen
NA	Not assigned
NMDS	Non-metric multidimensional scaling
NTC	Non-target controls
MU	4-methylumbelliferone
OA	Organic amendment
OQDS	Olive quick decline syndrome
P	Phosphorus
PAH	polycyclic aromatic hydrocarbons
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PGPB	Plant growth-promoting bacteria
rRNA	Ribosomal ribonucleic acid
SMS	Spent mushroom substrate
spp.	Species pluralis
TNb	Total nitrogen bound
TRIS	2-Amino-2-(hydroxymethyl)propane-1,3-diol
TP	Transitional phase
UAV	Unmanned aerial vehicle
V.	Version
VOC	Volatile organic compounds

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1 Introduction

1.1 Growing World Population and Climate Change

According to projections of the FAO (2009) the world's population will reach 9.1 billion people by 2050. Since this increase will mainly occur in developing countries, concomitant with increasing income levels and social prosperity, it is estimated that the rising demand on resources will require 70 % more food and feed production worldwide (FAO, 2009; Hunter et al., 2017). The resulting rising anthropogenic activities together with the accelerated pace of urbanization (EEA, 2015) and increasing land degradation (e.g. soil erosion, contamination, acidification, salinization, desertification) will exert tremendous pressure on agricultural areas and cause a decline of fertile soils if no appropriate actions are taken (Cameron et al., 2013; Zia-ur-Rehman et al., 2016). Facing these increasing burdens for ensuring global food and feed supply and furthermore to contribute to the increasing demand of biomass as feedstock for the industrial production of bio-based products and renewable energies (e.g. liquid biofuels) (Rathmann et al., 2010) an improvement of the agricultural production and its subsequent utilization will be required (Schröder et al., 2018).

Moreover, climate change particularly interferes with agricultural production through more frequent climate extremes (e.g. extreme temperature, drought, heavy rainfall, flooding) and indirect impacts (e.g. pests, diseases, rise of the mean sea-level) which can cause a decrease of crop productivity and cultivable field areas (Gornall et al., 2010). In turn, agriculture itself is a driver for climate change by greenhouse gas emissions (e.g. carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4)) and reducing its sinks by sacrificing natural land (e.g. rainforests) for the expansion of crop fields (HLPE, 2012). Consequently, agricultural management needs to adapt to future climatic conditions and furthermore to mitigate its influence on climate change to counteract the exacerbation of the global conflict on ensuring food, feed and biomass supply. For this, agricultural research and policy has to develop and pursue mitigation strategies aiming to protect ecosystem services that reduce emissions and increase the storage capacity of greenhouse gases (e.g. improve nitrogen fertilizer management, carbon sequestration) (FAO, 2007; Gøtke et al., 2016). Besides, adaptation strategies (e.g. biological pest control, biodiversity conservation, pollination, water and soil protection) need to be implemented so that agricultural ecosystems better adjust to moderate harm and exploit beneficial mechanisms to cope with climate variations (Locatelli, 2016; Power, 2010).

In this context the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI), funded by the EU, aims to contribute to sustainable intensification of agricultural systems, ensure food security under climate change, and to find synergies and reduce trade-offs between food supply, biodiversity and ecosystem services (Gøtke et al., 2016). One important aspect herein is to counteract the increasing pressure on arable soils by decreasing the input of mineral fertilizer and other agrochemical compounds on agricultural fields and to restore valuable set-aside and depleted sites for agricultural production. For this purpose, the application of organic amendments to poor soils, the utilization of beneficial microbes and even the gentle conversion of neglected, marginal grassland have raised interest and have thus been addressed in the present work embedded in the FACCE-JPI project INTENSE. The project aimed to intensify agricultural production, to transform biomass to energy and novel goods and to protect soils on selected marginal sites across Europe, representing various problems (e.g. low productivity, water scarcity, inappropriate land use), with adapted management strategies (Schröder et al., 2018). To evaluate in a holistic way whether such agricultural management practices are sustainable and suitable to restore marginal land, it is essential to assess soil health, plant performance and microbial mechanisms with modern techniques, such as molecular barcoding and remote sensing. This will widen our knowledge about the plant-microbe interaction and furthermore provide information on how to contribute to an environmentally sound agricultural intensification aiming moreover to close production circles within a future circular bioeconomy.

1.2 The Nitrogen Cycle

One of the major components to ensure global agricultural production is nitrogen (N), since it is a fundamental component of living organisms and its availability is one of the dominant limiting factors for plant growth (Gruber and Galloway, 2008; Kuypers et al., 2018). Although nitrogen, in the form of dinitrogen (N_2), is the most abundant gas in Earth's atmosphere, its plant availability is limited (Stein and Klotz, 2016). It is thus essential to understand the mechanisms of the N-cycle, one of the major biogeochemical cycles, to provide plants with plant available inorganic forms of N, such as nitrate (NO_3^-) and ammonium (NH_4^+). An important role is attributed to microbial activities and enzymatic reactions that drive the transformation of inert N_2 to 'reactive nitrogen' which was traditionally separated into three processes – N_2 fixation, nitrification, and

denitrification (Stein and Klotz, 2016). However, the rapidly increasing knowledge based on novel molecular approaches and interdisciplinary collaborations has expanded the classical separation of these processes and suggests the term 'microbial nitrogen-cycling network' to be more suitable to describe the complex coherences of nitrogen-transforming reactions (Kuypers et al., 2018; Stein and Klotz, 2016). Among the novel identified reactions are the dissimilatory reduction of nitrite (NO_2^-) to nitric and nitrous oxides (NO and N_2O) ('nitrifier denitrification'), the dissimilatory reduction of NO_2^- to NH_4^+ ('respiratory ammonification'), and the dissimilatory oxidation of NH_4^+ ('anammox') (Stein and Klotz, 2016). Consequently, the 'nitrogen cycle' is now separated into major nitrogen-transforming flows that are dominated by ammonification, nitrification, denitrification, anammox, and nitrite-nitrate interconversion (Stein and Klotz, 2016). These nitrogen-transforming flows are essential for providing plant available nutrients but their processes are not balanced and associated with different nitrogen fluxes (Kuypers et al., 2018). These fluxes of soil N are amongst others affected by ammonia (NH_3) volatilization, NO_3^- leaching, mineralization, adsorption, and microbial N immobilization. In agriculture, they can be influenced by appropriate management (Ju and Zhang, 2017; Murphy et al., 2000). Moreover, even plants contribute to the production of plant available nitrogen forms, such as NH_3, which is well documented for the symbiosis of leguminous plants with root nodule forming nitrogen fixing bacteria (Dixon and Kahn, 2004). Recent discoveries furthermore highlight the role of root exudates and their influence on soil nitrification, stimulation of root nodulation, and nitrogen fixation even in neighboring plants and thus on shaping nitrogen flows (Coskun et al., 2017). However, nowadays the industrial fixation of atmospheric nitrogen (N_2) (e.g. Haber-Bosch process) and other anthropogenic sources (e.g. combustion of fossil fuels) are dominating terrestrial nitrogen flows (Fowler et al., 2013; Stein and Klotz, 2016).

1.3 Mineral Fertilizer and Conventional Agriculture

Since more than one century the invention of the Haber-Bosch process facilitates the industrial production of ammonia (NH_3) from atmospheric nitrogen gas (N_2) and thus the commercial application of synthetic N fertilizer onto arable fields (Erisman et al., 2008). The so-called Green Revolution of the 20th century facilitated an intensified and expanded agricultural production with increasing crop yields, boosting the growth of the world population and contributing to its raising

demand (Stein and Klotz, 2016). Unfortunately, the increasing production of mineral N fertilizers was accompanied by high energy consumption which, in case of production with non-renewable energy, leads to high amounts of emitted greenhouse gasses (e.g. CO_2), thus raising the pressure on our climate (Fischedick et al., 2014; IFA, 2009). In addition, also increasing N_2O emissions following an excessive application of N fertilizer on arable fields exacerbate the climate conflict since they harm the ozone layer (Cameron et al., 2013). To date, agricultural soils are the largest emitters of N_2O due to microbial reduction and oxidation of nitrogen (Cavicchioli et al., 2019).

Besides high energy consumption and greenhouse gas emissions, excessive application of mineral fertilizer and other conventional management practices (e.g. addition of agrochemical compounds, intensive tillage etc.) affect soil, plant, animal, and even human health (Horrigan et al., 2002, Timsina, 2018; Wall et al., 2015). With its project calls, FACCE-JPI aimed to counteract these detrimental developments. Both, short- and long-term application of mineral N fertilizers are known to reduce soil quality, its taxonomic diversity and to change the microbial composition in grassland and arable soils (Geisseler and Scow, 2014; Pan et al., 2014; Semenov et al., 2020; Van Zwieten, 2018). Since soil microbial diversity is crucial for providing multiple essential soil functions and thus affects plant health it is further important for the quantity and quality of food production and thus human health (Tsiafouli et al., 2015; Wall et al., 2015). Moreover, increasing land use intensities have been reported to reduce plant species richness, species richness of the soil fauna and its activities as well as to promote a decline of diversity and complexity of soil food webs in different agricultural regions across Europe (Kidd et al., 2017; Singh et al., 2018; Tsiafouli et al., 2015). The excessive application of mineral N fertilizer together with the associated nutrient losses furthermore leads to unprecedented environmental pollution and land degradation (Stein and Klotz, 2016) like groundwater contamination, marine eutrophication, soil depletion and soil acidification (Cameron et al., 2013; Horrigan et al., 2002). The acidification of arable soils following increasing H^+ concentrations (caused by mineral N fertilization) can in turn negatively impact plants and other organisms (Crews and Peoples, 2004; Hao et al., 2020; Kidd et al., 2017). The undesirable accumulation of NO_3^- in groundwater, roots, vegetables, fish, processed food and many other components of the human food chain and their later consumption furthermore contributes to endogenous nitrosation which can affect human health (e.g. diabetes and thyroid disorders) and ultimately even might lead to cancer (Ahmed et al., 2017; Hansen et al., 2017). It is estimated that 38 % of water bodies within the European union are significantly under constraint

from diffuse agricultural pollution (WWAP, 2015) which in turn causes stricter restrictions to farmers since e.g. fertilizer legislations, such as the European Nitrate Directive (Directive 91/676/EEC), are facing this problem to protect water resources from agricultural pollution (Hansen et al., 2017).

Hence, it is crucial to face the negative consequences of conventional farming and to achieve more sustainable agricultural production which focuses not only on high crop production but also on protecting soil quality, soil functionality, soil health (Lal, 2016) as well as atmosphere, lithosphere and hydrosphere.

1.4 Organic Amendments

To address the negative aspects of the excessive use and production of mineral N fertilizer, the application of organic amendments (OA) is worldwide raising interest (SCAR-report, 2015). Since it is known that organic amendments comprise a great variety of positive but also negative effects on soil health and plant performance these will be discussed in the following section (Gómez-Sagasti et al., 2018; Schröder et al., 2018; Urra et al., 2019).

1.4.1 General Advantages of Organic Amendments

The consideration of farm wastes and organic residues as treasure of valuable resources for future fertilization strategies is one of the key factors for sustainable agricultural farming. Although the application of e.g. compost, plant residues and animal manure to maintain soil fertility and improve plant production is not novel and can be traced back to at least the 3rd millennium BC (Van Zwieten, 2018), modern research rediscovers its wide range of opportunities (Schröder et al., 2018). Such organic amendments can be obtained directly from the farm or produced via cascading, upgrading and recycling of bio-based products, and influence the physical, chemical, and biological properties of soil differently (SCAR-report, 2015; Schröder et al., 2018). Improved soil physical fertility, mainly because of enhanced aggregate stability and reduced bulk density has been reported together with improved crop performance after long-term application of organic residues (Diacono and Montemurro, 2011). These authors furthermore observed increased water holding capacity, reduced soil erosion and NO_3^- leaching after repeated application of composted materials. Moreover, the chemical properties of organic amendments, which are usually rich in

nutrients and organic matter, are beneficial for arable and depleted soils and even facilitate atmospheric carbon sequestration (Diacono and Montemurro, 2011; Odlare et al., 2011). Besides, the amendment's biological properties, in particular its microbial composition, activities and abundance of specific microbes, are known to improve soil quality, plant health and crop production, for instance, via enhanced nutrient cycling and better suppression of soil-borne diseases (Bonilla et al., 2012; Odlare et al., 2011). Scotti et al. (2015) found organic amendments a sustainable tool to recover soils previously depleted by intensive agriculture. Lori et al. (2018) reported a more stable N provisioning potential even under future drought scenarios for organically managed soils which might help plants to adapt to future climate extremes. However, in most cases organic farming is still expected to produce lower yields than conventional farming (Timsina, 2018) although it is considered to be more profitable, environmentally friendly and likely to provide more ecosystem services and better food (Reganold and Wachter, 2016).

Nevertheless, since the characteristics of organic amendments vary greatly, the individual properties of different soil amendments need to be considered when searching for suitable fertilizers to enhance agricultural crop production on different soils.

1.4.2 Spent Mushroom Substrates and Digestates

An interesting organic amendment originating from farm activities consists of spent mushroom substrates (SMSs) which are obtained in large quantities as by-product from industrial mushroom production (e.g. *Agaricus bisporus, Pleurotus* spp.) (Hanafi et al., 2018; Paula et al., 2017). Regional producers of mushrooms depend on the delivery of straw, composts, and other farm materials as substrates for mushroom cultivation, and have high amounts of the used substrate left after harvest. The areas of SMS-application range from enzyme production, bioremediation, wastewater treatment, animal feeding up to crop production (Hanafi et al., 2018; Phan and Sabaratnam, 2012). Its reutilization lowers the amounts of residues and wastes within the food industry and thus contributes to circular bioeconomy (Grimm and Wösten, 2018). In terms of crop production, the favourable physical properties, the richness in complex organic matter and the high nutrient contents (e.g. nitrogen (N), phosphorus (P), and potassium (K)) (Paula et al., 2019) of SMSs are promising for plant-growth promotion especially if they are stabilized trough composting (Paula et al., 2017). Different studies were able to prove beneficial effects on soil structure and microbial

abundance together with improved crop production after SMS application on arable fields (Paredes et al., 2016; Paula et al., 2019). However, depending on their composition it might be beneficial to combine SMSs with other organic or inorganic amendments to improve their physical, chemical, and biological properties and thus their capability for enhancing crop yield. For this purpose, the SMS used within this study was blended with digestate (30 %) and straw (20 %) during the composting process and subsequently pelletized for further improving storage and transportation capabilities. Interestingly, SMSs are also described as promising candidates for nursery seedling growing media and recommended to replace the expensive and resource-limited peat in greenhouse cultivation (Paula et al., 2017; Zhang et al., 2012). However, prior to its application on arable fields the treatment of SMS (e.g. desalting, composting) is advisable, and its physical, chemical and microbial properties need to be monitored (Ahlawat and Sagar, 2007; Paula et al., 2017).

Biogas digestates, residues from anaerobic fermentation of organic matter, comprise another interesting soil amendment for organic farming since they contain valuable nutrients and can be used to close agricultural nutrient cycles (Ehmann et al., 2018). Tambone and co-workers (2010) recommend digestates due to their very good fertilizing properties caused by highly plant available nutrients (N, P, K) and considerable amounts of organic carbons. Nabel et al. (2017) found digestates beneficial to increase plant performance and improve soil fertility especially of marginal soils by increasing their soil carbon content, water holding capacity and basal soil respiration. From such type of amendments, Odlare and co-workers (2011) achieved crop yields almost as high as from mineral fertilizer together with improved microbiological properties like potential ammonium oxidation, substrate induced respiration and nitrogen mineralization. It is thus, why many studies found digestates suitable to replace mineral fertilizer while maintaining agricultural productivity and reducing nutrient leaching (Odlare et al., 2011; Tambone et al., 2010; Walsh et al., 2012). Consequently, the application of digestates, preferably originating from the vicinity of the farm, on arable fields lowers the impact of agricultural measures on the environment and reduces wastes of the biological production of methane-rich biogas for electricity, fuel and heat generation which replaces the usage of fossil energy. In short, amendments containing digestates aid to implement a circular bioeconomy.

1.4.3 Biochar

Biochar, another promising soil amendment, is recently raising interest around the globe, even though it is already in use since thousands of years originally associated with soils of the Amazon region (Panwar et al., 2019; Qambrani et al., 2017). Biochar obtained from pyrolysis under low to mid oxygen supply and high temperature (Lehmann et al., 2011) can influence soil fertility mainly through enhancing physical soil properties (e.g. soil structure) and soil nutrient pools (Panwar et al., 2019). Whether soil physiochemical properties following its application change and promote microbial colonization strongly depends on its physical (e.g. pore size, pore volume, surface area) and chemical properties (e.g. pH, nutrient content) (Lehmann et al., 2011; Palansooriya et al., 2019). Increased soil microbial biomass, enhanced microbial metabolic activities and changes of microbial composition after biochar application have been observed and promote soil health and plant performance (Palansooriya et al., 2019; Zhou et al., 2019). Biochar is extremely rich in organic carbon and shows high potential in regulating soil nitrogen flows through enhancing symbiotic biological N_2 fixation, improving plant N uptake and decreasing nitrogen leaching (Haider et al., 2017; Liu et al., 2018b; Panwar et al., 2019; Ulyett et al., 2014). Moreover, improved water retention, enhanced pesticide degradation due to its high surface area (caused by its porous microstructure), and the restoration of multiple soil functions have been reported (Ohsowski et al., 2012; Ding et al., 2017). Biochar application furthermore enables atmospheric carbon sequestration (Matovic, 2011) and even reduces N_2O emissions (Liu et al., 2018b; Panwar et al., 2019), thus counteracting anthropogenic greenhouse gas emissions. The production and application of biochar might mitigate the agricultural influence on climate change, improve waste management in a circular bioeconomy while it releases energy during its pyrolytic production (Panwar et al., 2019; Qambrani et al., 2017). Nevertheless, since feedstock, pyrolysis temperature, application rate, particle size and reactor conditions have great influence on the characteristics of biochar more studies are required to quantify its suitability to restore soil functions, improve plant growth and sequester carbon (Agegnehu et al., 2017; Ding et al., 2017; Qambrani et al., 2017). In addition, although using the same soil and biochar in field and greenhouse studies, Haider et al. (2017) found contrasting results which supports the urgency for more investigations to confirm the potential of biochar to improve soil quality and plant performance. In the present study, the farmer had invested in a pyrolysis reactor to yield heat from plant residues of his farm, and to produce biochar for field application.

1.4.4 Negative Aspects of Organic Amendments

Despite multiple advantages of organic amendments also negative effects can arise already during their production or after their application on arable fields. This is why, first the emission of greenhouse gases that are produced during composting, digestion or pyrolysis need to be minimized to improve the amendment's sustainability (Schröder et al., 2018). Subsequently, its composition needs to be monitored and optimized to minimize negative consequences after field application. For example, organic amendments derived from arbitrary composts might contain a variety of environmental pollutants such as heavy metals (Madrid et al., 2007), persistent organic pollutants and potential human pathogens (Urra et al., 2019) which might of course be enriched especially when using compost from urban feedstock compared to rural input material (Brändli et al., 2005; Odlare et al., 2011). Moreover, significant amounts of (micro)plastic, which is raising global awareness due to its ubiquitous occurrence also in the aquatic environment (Barceló and Picó, 2018), have been detected in soils amended with municipal compost (Watteau et al., 2018). Similarly, the previously mentioned biochar is known to contain residual pollutants as by-product of its pyrolytic production like polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs) and carbon nanoparticles which may affect soil microbes and plant growth negatively if not selected properly (Dutta et al., 2016). At last, application of organic fertilizers (e.g. animal manure) can moreover introduce emerging pollutants such as antibiotic residues, antibiotic-resistance genes and antibiotic resistant bacteria on arable fields which is currently receiving global attention since it is of concern for public health (Gómez-Sagasti et al., 2018; He et al., 2016; Urra et al., 2019).

1.4.5 Optimizing Production and Composition of Organic Amendments

It is hence crucial to monitor physical, chemical and biological properties of soil amendments prior to their application on arable fields and to optimize the processes involved in their production. The addition of organic, inorganic or biological additives during composting, for instance, can be used to buffer pH, regulate its moisture and temperature, improve aeration and even to stimulate microbial activity which helps to improve the composting process and finally to optimize the quality of the amendment (Barthod et al., 2018). This affects its later impact on soil ecosystem services, such as improved soil fertility and carbon sequestration, and moreover helps to minimize greenhouse gas emissions already during its production

(Barthod et al., 2018). As an example, the addition of pig manure to SMS and rice husks has been shown to enrich the nutrient content, bacterial diversity and anti-pathogens (e.g. *Pseudomonas* spp.) of the final compost and even accelerates its maturity (Meng et al., 2018). Moreover, addition of biochar during composting can be used to reduce heavy metal mobility (Barthod et al., 2018) and to increase the amendments´ C/N ratio which subsequently impacts soil microbial composition and activity and thus mineralization processes and plant N uptake (Heijboer et al., 2016). In general, increasing microbial N immobilization and soil N retention for organic amendments with high C/N ratios as well as stimulation of plant growth in case of low C/N ratios have been reported (Heijboer et al., 2016). Thus the physical, chemical and biological compositions of soil amendments can influence soil microbes and functions which hence need to be analyzed aside from soil nutrient stocks and plant performance to assess the effects after organic amendment application as a whole.

We found the co-composting of SMS with digestate and straw to be a promising candidate for a composed organic amendment of high quality. Its positive impact on soil health and plant performance might even be improved when combining it with other amendments before applying it onto arable fields. Therefore, the combination with biochar and even inorganic N fertilizer may be considered to act synergistically (Stewart et al., 1998; Timsina, 2018). Thus, our greenhouse study (M2) aimed to evaluate effects after application of various compositions of the mentioned SMS-pellets together with biochar and mineral fertilizer on soil functions, plant performance and soil-rhizosphere microbiota during barley growth.

1.5 Microbial Composition in Soil and Influence on Plant Growth

As already indicated, the microbial composition and activity in soil is extremely complex and provides important soil functions that are influencing a large number of key processes which can have strong positive but also negative impact on plant performance (Barrios, 2007; Van Der Heijden et al., 2008). This will be elucidated in the following chapter.

1.5.1 Positive Aspects of Microbes for Soil and Plant Growth

Microbes are the major drivers of multiple essential soil functions, such as the biochemical carbon and nitrogen cycles, and thereby affecting soil, plant, animal and even human health (Falkowski et al., 2008; Cavicchioli et al., 2019). The microbial mineralization of nutrients into plant available forms is crucial for plant performance and has been explained above exemplarily for the N-cycle (Barrios, 2007; Kaiser et al., 2016). The probably most prominent example herein are N-fixing rhizobia primarily found within root nodules of leguminous plants. It is estimated that the amount of N fixed by rhizobia is almost as high as that from synthetic NH_3 production (Gruber and Galloway, 2008) which highlights its significance for plant growth-promotion. Besides their ability to biologically fix atmospheric N these rhizobacteria are able to convert N_2O to inert N_2 through the enzyme N_2O-reductase and thus additionally help to mitigate greenhouse gas emissions from agricultural fields (Cavicchioli et al., 2019). Other plant growth-promoting bacteria (PGPB), such as some members of *Pseudomonas* spp., *Burkholderia* spp., and *Rhizobium* spp., are known to protect plants against diseases and abiotic stress and support plant performance through multiple further mechanisms (Barrios, 2007; Preston, 2004; Souza et al., 2015). Some members of the genus *Pseudomonas* spp., for instance, are known to protect several major agricultural crops from diseases by the production of antifungal metabolites which may moreover reduce the requirement of fungicides on arable fields (Preston, 2004; Weller et al., 2002). PGPB furthermore are capable to solubilize phosphate, increase ACC deaminase activity, and produce siderophores and phytohormones and thus increase agronomic efficiency when used as bacterial inoculant (Souza et al., 2015). Hence, Glick (2012) stated that in the not too distant future, the application of PGPBs will begin to replace the array of chemicals used within agricultural systems. Nevertheless, it will remain important to consider also the prevailing soil microbiota for the evaluation and preparation of arable fields since it provides essential soil functions (Fernandez et al., 2016) and, amongst others, has great potential to help plants (e.g. barley) to

cope with abiotic stress (e.g. drought) through the recruitment of soil-borne endophytes (Yang et al., 2020). In general, it is known that the microbial composition and activity in soil provides a great variety of important ecosystem services which are essential for a sustainable management of agricultural ecosystems and thus ensures agricultural productivity (Barrios, 2007; Van Der Heijden et al., 2008).

1.5.2 Negative Aspects of Microbes for Soil and Plant Growth

In contrast, also negative aspects of microbes are known to have strong impact on soil health and plant performance which may have dramatic consequences for agricultural productivity. Important examples are microbial pathogens like members of *Fusarium* spp., *Pythium* spp., and *Phytophthora* spp. which are known to cause serious plant diseases like fusarium wilt, root rot or potato late blight with the latter known to be responsible for the great Ireland's potato famine in the 1840's (Bodah, 2017; Ristaino et al., 2013; Van Der Heijden et al., 2008). Nowadays, the species *Xylella fastidiosa*, that causes the so called olive quick decline syndrome (OQDS) which leads to the desiccation of e.g. olive trees, concerns farmers in the Mediterranean and around the globe (Saponari et al., 2019; Sicard et al., 2018). Moreover, some members of the previously mentioned *Pseudomonas* spp. are known to inhibit plant growth and cause disease symptoms (e.g. rot and necrosis) through the development of dystrophies (e.g. galls) (Preston, 2004). In strongly nutrient limited soils, microbes can compete with plants for available nutrients which can negatively impact plant nutrient acquisition and growth (Van Der Heijden et al., 2008). Another disadvantage follows the transformation of NH_4^+ to NO_3^- through nitrifying bacteria since NO_3^-, as the more mobile fraction in soil, will leach easier into groundwater with the already mentioned negative consequences (Van Der Heijden et al., 2008).

Altogether, this great variety of positive but also negative effects highlights the importance of microbial mechanisms for maintaining soil health, providing soil functions, improving plant performance and thus to ensure global food, feed, and biomass production.

1.5.3 Parameters that Influence Microbial Composition and Activity

Since, accordingly, soil microbes are pivotal for agricultural productivity, it is crucial to know which parameters are shaping the microbiome´s composition and activity and thus affect soil health and plant performance (Herzog et al., 2015). This is particularly of interest in organic

farming systems, since due to the lack of synthetic fertilizers agricultural productivity depends strongly on biological soil processes, especially on nutrient cycling and turnover of organic material (Fernandez et al., 2020). Many studies found soil pH as major driver of the microbial composition and its activities in soil followed by other important soil characteristics like its texture, moisture, and temperature (Cao et al., 2016; Fernandez et al., 2020; Kaiser et al., 2016; Lauber et al., 2009; Shen et al., 2013; Wu et al., 2017). However, also vegetation cover, land use type and intensity, plant species, soil nutrient stocks (e.g. carbon, nitrogen) and their ratio are known for their great impact on shaping soil microbial composition and activity (Drenovsky et al., 2010; Estendorfer et al., 2017; Shen et al., 2013; Yang et al., 2017). Besides, soil bacteria can be affected by climatic conditions, especially by temperature and precipitation, with the latter being more important for affecting soil and also plant bacteria (Sheik et al., 2011; Yuan et al., 2014). It is thus essential to additionally consider future climate scenarios, especially with regard to climate extremes (e.g. drought, flooding), when aiming at the restoration of depleted agricultural soils and its associated microbiota. However, multiple studies found that temperature and precipitation might have weaker influence on the bacterial composition in soil than the agricultural management and soil characteristics (Drenovsky et al., 2010; Hermans et al., 2017; Xue et al., 2018).

1.5.4 Influence of Organic Amendments on Microbial Composition and Activity

As already described, agricultural management practices, such as the application of organic amendments, are known for their influence on soil microbial composition, and microbial and enzymatic activities (Albiach et al., 2000; Bandick and Dick, 1999; Bonilla et al., 2012; Schmid et al., 2017). Changes of the soil microbial composition after amendment application have been reported to influence microbial N immobilization, soil N retention, and plant N uptake which also affects plant performance (Heijboer et al., 2016). Moreover, increasing microbial and enzymatic activities, related to the incorporation of organic matter rich amendments, were found to stimulate multiple important soil functions such as nutrient mineralization (Heijboer et al., 2016; Zhong et al., 2010). For example, rising soil extracellular enzyme activities (EEA) after amendment application were reported to promote the degradation of complex organic compounds to lower molecular weight substrates like sugars, amino acids or ammonium which are essential for plant nutrition (Burns and Dick 2002; Allison and Vitousek 2005). A case example are SMSs which usually comprise a high proportion of slowly decomposable

lignocellulosic waste (Hanafi et al., 2018) and consequently high abundances of carbon-degrading microbes (like *Bacillaceae* and *Thermomyces*) which finally affect microbial composition and activities in soil (Meng et al., 2018). Hence, besides their potential to provide essential nutrients, organic amendments further need to be analyzed with regard to their microbial composition, microbial and enzymatic activities and their impact on soil microbiota prior to their application onto arable fields.

Of course, it is important in this context to distinguish the different compartments of natural and agricultural ecosystems which are usually affected by agricultural management such as amendment application. The main separation can be found between bulk soil and the plant's rhizo- and endosphere. A comparison of rhizosphere vs. bulk soil shows denser bacterial population, increased microbial activities, and higher turnover rates which highlights its importance for nutrient cycling and improving plant performance (Vieira et al., 2019). However, the interaction of these compartments is crucial since the majority of plant-associated bacteria derives from bulk soil but, as shown for PGPBs, first need to migrate to the rhizosphere to unfold their beneficial effects (Compant et al., 2010). Thus, understanding plant colonization processes of bacteria is essential to foster their influence on plant rhizo- and endosphere. This is especially of interest when beneficial bacteria are intended to be used as inoculants for biofertilizers or biocontrol agents (Hegazi et al., 2019).

Overall, this chapter demonstrates the importance of understanding microbial mechanisms for improving agricultural production while also maintaining soil health. This is furthermore of interest when management strategies are developed to recover depleted soils or to unlock the potential of marginal sites and to adapt sustainable cropping systems to future climate scenarios.

1.6 Converting Marginal Sites

Due to the initially described intensification of the pressure on fertile soils, it will furthermore be indispensable to upgrade marginal land that has so far not been considered for agricultural production (Schröder et al., 2018). In the past, such land was set-aside since the expected yields were regarded too low or the management too difficult. It is thus essential to promote the exploitation of knowledge in upgrading unused and fallow land to enable a gentle land conversion and to alleviate the arising conflict between food, feed and biomass production (Harvey and Pilgrim, 2011).

When converting marginal land into high-quality or at least into productive land, the site-specific weaknesses and problems of any given location must first be assessed and defined. Subsequently, site-adapted smart agricultural management needs to be implemented to improve the capability of the soil to provide essential soil functions for crop production. This requires knowledge to be gained for future sustainable agricultural systems, especially with regard to appropriate organic amendments, land conversion and effects of climate change. The GREENLAND project has developed a simple and transparent decision support tool (DST) to find suitable gentle remediation options (GRO) particularly for contaminated sites (Cundy et al., 2015). As an example, phytomanagement with organic amendments and green manure application can be used to restore soil health and quality of degraded soils and can be embedded in a circular economy (Fageria, 2007; Gómez-Sagasti et al., 2018). If such sustainable and gentle management of marginal sites and depleted soils is well planned, soil structure and thus fertility can be increased again (Vanlauwe et al., 2010). Wall et al. (2015) found maintained and partially even restored soil biodiversity and ecological complexity when improved management practices were used even on formerly intensively used sites. Moreover, the authors stated that even human health was improved when soil was managed sustainably. In consequence, after the development of suitable site-adapted management strategies, it may be a viable option to convert marginal sites into arable land.

In this context even the conversion of poor, marginal grassland, which was neglected and set-aside in the past, should be considered, if the transition to economically attractive crop land can be achieved in a sustainable manner. Of course, it is essential to monitor such conversion and optimize the transition strategy since it is known that the intervention in such ecosystems can change and negatively impact microbial diversity and its composition in soil (Gatica and Cytryn, 2013,

Carbonetto et al., 2014, Hartmann et al., 2015). Furthermore, the grassland break-up, in the beginning of the conversion, will likely cause other negative effects like increased N losses due to accelerated NO_3^- leaching as well as increased N_2O emissions due to decomposition of grass residues and accelerated mineralization of soil organic N (Buchen et al., 2017). Thus, it will be essential to compensate these negative consequences and to optimize the conversion strategy gently while implementing sustainable farming practices. For this, soil nutrient stocks need to be stabilized and the prevailing microbial composition and activities have to be conserved or even enhanced.

In this context, leguminous plants which are well known for their capability to fix atmospheric N and their influence on shaping nitrogen flows are raising interest (Torabian et al., 2019). The intercropping of legumes as cover crop seems to be a promising method for not only improving soil fertility due to atmospheric N fixation but furthermore to reduce nutrient leaching and improve water retention (Fan et al., 2006; Plaza-Bonilla et al., 2015; Stagnari et al., 2017). The additional release of high-quality organic matter into the soil together with the capability to sequester carbon further highlights the great potential of legumes when used as cover crop and crop residue (Plaza-Bonilla et al., 2015; Stagnari et al., 2017). Due to the multiple beneficial effects we selected *Vicia faba* L., a prominent member of the *Leguminosae/Fabaceae* family, during the conversion of the marginal grassland, under consideration to enrich soil nutrient stocks, avoid invasion of undesired weed species and to prevent nutrient leaching, as strategy for a sustainable and gentle preparation of an arable land (M1). Besides improved nitrogen use efficiency (NUE) and plant performance this might moreover reduce pest problems, requirements for agrochemicals and stimulate biodiversity which has been already shown for intercropping of legumes and cereals (Duchene et al., 2017; Jensen et al., 2020).

However, since little is known about the influence of intercropping legumes during grassland conversion and its later incorporation on microbial composition and activities it must be monitored to provide farmers and decision makers with essential knowledge to give appropriate instructions for future measures.

1.7 Smart Management with Advanced Technologies

Besides the application of organic fertilizers, the conversion of marginal land and the optimization of beneficial plant-microbe interactions the transformation from conventional to sustainable agriculture must include practices of precision agriculture to address site heterogeneity (Schröder et al., 2019). Here, especially the non-destructive collection of data is promising, to assess individual properties of agricultural sites, monitor land use changes and to lower the input of fertilizer and other agrochemical compounds (Schröder et al., 2018). For instance, hand held devices (e.g. SPAD, Dualex 4) used for the estimation of plant chlorophylls, which can be correlated to the leaf nitrogen content, can help to improve nitrogen management especially on small-scale farms (Cerovic et al., 2012; Yue et al., 2019). Moreover, vehicle borne sensors (e.g. EM38) are widely used to assess different soil parameters like its moisture and thus help to characterize agricultural areas (Heil and Schmidhalter, 2019). However, especially remote sensing with unmanned aerial vehicles (UAVs) equipped with RGB and multi-spectral cameras is raising interest worldwide since it ensures high spatial and temporal resolution and the images can be captured quickly, inexpensively and without great effort (Jin et al., 2009; Zhang and Kovacs, 2012). The captured spectral responses can then be used to calculate various indices indicating multiple plant and soil parameters. Besides the analysis of soil properties (Ge et al., 2011), the detection of compaction and inhomogeneity in fields, even the localization of erosion effects and land degradation is possible (d'Oleire-Oltmanns et al., 2012; Turner et al., 2015; Krenz et al., 2019) and fosters rapid field characterization. Furthermore, remote sensing enables the evaluation of plant health and plant performance (Bendig et al., 2015; Candiago et al., 2015; Rueda-Ayala et al., 2019; Thenkabail et al., 2000), leaf are index (LAI) in the canopies as well as macronutrient deficiencies (e.g. N, K) (Haboudane et al., 2004; Nigon et al., 2015; Severtson et al., 2016; Barbedo, 2019) which facilitates the optimization of fertilization strategies. Since pictures can even be processed in the field using a laptop, remote sensing grants a rapid response application and actual time detection. Using this methodology, farmers and decision makers can plan more targeted and efficient application of fertilizers and other agrochemical compounds to optimize their management strategy already on the field which will help to mitigate the pressure on arable soils.

1.8 Aims of the Thesis

The present thesis is embedded in the INTENSE project which was supported and funded by the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI) of the European Research Area (ERA-NET). In this framework it aims to intensify agricultural production and to transform biomass to energy and novel goods and to protect soils in selected European countries. Within INTENSE, the focus of this thesis is to identify responses of soil nutrient stocks and bacterial community structure following (**I**) the conversion of neglected and marginal grassland in southern Bavaria, (**II**) the application of different combinations of organic amendments and mineral fertilizer in a short-term *H. vulgare* greenhouse experiment, as well as (**III**) summarizing the state of the art of European agricultural visions how to mobilize marginal lands.

Objectives

The specific objectives of the present work were to elucidate the responses after an optimized management strategy for the conversion of a neglected grassland and the effects of organic amendments and mineral fertilizer on soil nutrient stocks, bacterial community structure, and plant performance. In more detail, objectives and different experimental parts aimed to restore marginal land and comprised:

(I) Monitoring effects during the conversion of marginal grassland to arable land via a transitional nitrogen fixing phase with respect to soil nutrient stocks and microbial composition. In focus were the changes caused by mineralization following the grassland break-up, the biological nitrogen fixation of *V. faba* plants, and processes following the incorporation of the grass and leguminous residues into the soil.

(II) Identifying microbial mechanisms after application of different organic and mineral amendment combinations in a *H. vulgare* short-term greenhouse experiment. The focus was to analyze whether the input of organic amendments improves plant performance and maintains soil quality, if its application with and without mineral fertilizer will

change microbial activity, bacterial composition and potential extracellular enzyme activity differently as well as if the C/N ratios of the different fertilizer compositions will impact the microbial N immobilization.

(III) Providing a toolbox for transforming marginal land into productive agricultural land including different scenarios from the INTENSE project. Besides the role of crops and organic amendments on marginal soils, different aspects of the plant-microbe interaction and its influence on plant health and performance should be elucidated. The importance of indicators and models for land use planning were highlighted to provide farmers and decision makers with knowledge for future sustainable cropping systems and to unlock the potential of marginal lands.

As such the project results are directly influential to the work of the involved farm, and will determine the future production at the selected site. Furthermore, remote sensing as a 'novel' tool for a relatively affordable and more precise agriculture to improve agricultural sustainability was used.

2 Material and Methods

2.1 Study Site Description

The experimental site to analyze responses induced by the conversion of marginal grassland to arable land (M1) is based at Martlhof in Ostin am Tegernsee (Bavaria, Germany, 47° 44' 37.30" N and 11° 45' 38.32" E). Soil for the study of the effects of organic and inorganic amendments during barley cultivation in a greenhouse (M2) was also excavated from this experimental site.

The traditional small-scale dairy farm is located 784 m above sea level and the experimental field (1 ha) with a gently sloping relief was formerly used as extensive grassland where sheep and pigs were raised on pasture. The climatic conditions of this region, in the Bavarian alpine upland, are characterized as the transition zone of the warm-temperate climate of Western Europe and the colder continental climate of Eastern Europe. Over the last 30 years, a mean annual precipitation of 991 mm, a mean annual temperature of 7.5 °C, and a mean annual sunshine duration of 1571 h was observed. The bedrock of the site is calcareous and the colluvial topsoil contains 28.2 % sand, 43.1 % silt, and 28.8 % clay. Thus the texture was classified as clayey loam with an average pH ranging from 5.2-5.6. The land owner considered this plot as unproductive marginal grassland without value for his farm. He was seeking a new production line for his plot that would support his idea of small cattle and chicken production with on-farm feed production.

2.2 Experimental Layouts

2.2.1 Conversion of Grassland (M1)

A short-term field trial was started in May 2016 to investigate the impact of converting a marginal grassland to arable land on soil nutrient stocks and soil bacterial composition. From the beginning of the experiment, the experimental area (32 x 32 m) as a whole was subdivided into six 10.7 x 14 m sub-plots (see Appendix M1, Figure S1). For the independent replication and controls, the six subplots (I, II, III, IV, V, and VI) were separated and supplemented by four untreated grassland plots of 8 x 4 m each (Control). To assess the individual properties of the initial grassland a phytosociological description was performed according to Ellenberg (1992). The inventory of the grass species and their associated indicator values (e.g. individuality, sociability, temperature, and

nitrogen) are shown in Appendix M1, Table S1. At the beginning of the conversion, the initial grass scar was mechanically mulched and the residual green cover was incorporated into the soil. Subsequently, milling of the topsoil was done at a depth of 12 cm and broad beans (*Vicia faba* L.) were sown uniformly as cover crop with a density of 200 seeds/m². The aim was to homogenize the initial situation of the experimental field area, to enable biological fixation of atmospheric nitrogen, and at the same time to prevent undesired invasion of weeds and leaching of nutrients. The leguminous plant residues were incorporated in April 2017, and in May a three-furrow turning plough was used to till the topsoil up to a depth of 18 cm. The field reached its final state of the conversion after milling with a harrow.

2.2.2 Organic Amendments and Mineral Fertilizer in the Greenhouse (M2)

To analyze the effects of various compositions of organic amendments and mineral nitrogen fertilizer on barley growth and its associated bacterial composition in the soil-rhizosphere mixture a short-term greenhouse experiment was conducted. Soil was excavated at a depth of 20 cm from unfertilized plots one year after the final state of the previously described conversion experiment (M1).

The organic amendments studied in this short-term greenhouse experiment were pelletized spent mushroom substrate (SMS) blended with digestate and straw (P) with varying amounts of biochar (B) (see Table 1). The pellets were produced via conventional composting of 50 % SMS, combined with 30 % biogas residues from biogas production (digestate) and 20 % straw at a temperature of 59 °C and a compost humidity of 20-23 % (Prof. Szulc, Warsaw University of Life Sciences, Poland). The SMS was obtained after cultivation of *Agaricus bisporus* (JE Lange) Imbach from an industrialized mushroom farm in Poland. Biochar was produced at Martlhof via pyrolytic reaction (800 °C) of chopped pruning residues of local chestnut, beech, spruce, maple and ash trees. N_{tot} and C_{tot} content was 0.2 and 71.2 %, respectively. The final product had a pH of 8.5 ± 0.1, organic matter 91.9 ± 5.0 % and dry matter 81.8 ± 2.4 % content (personal communication, Prof. Maestri, University of Parma, Italy). A stimulating effect on germination and growth on *H. vulgare* was observed. No phytotoxic effect could be monitored.

Table 1 Chemical composition (N_{tot}, C_{tot}, and C/N ratio) of the organic amendments studied in the short-term greenhouse experiment with barley (*H. vulgare*). Shown are data for pellets (P), biochar (B), and combinations of pellets with 10 % biochar (PB10), and pellets with 20 % biochar (PB20).

	Organic amendment	**N_{tot} [%]**	**C_{tot} [%]**	**C/N**
P	Pellet	1.48	21.28	14
PB10	Pellet + 10% Biochar	1.46	25.28	17
PB20	Pellet + 20% Biochar	1.32	30.21	23
B	Biochar	0.23	71.19	310

In total thirteen compositions of pellets, biochar and mineral fertilizer were examined with focus on bacterial composition, potential enzyme activity, functional diversity and basic soil and plant properties. The initial soil contained 60 kg N ha^{-1} and was fertilized up to 200 kg N ha^{-1}. With respect to effects caused by differences in carbon and nitrogen contents the amendments were applied to reach equal C_{tot} and a maximum fertilization of 140 kg N ha^{-1}, except for treatments PB10N and PB20N (see Appendix M2, Table S1). For these treatments 140 kg N ha^{-1} were applied although higher C_{tot} contents than in the other treatments were reached. In addition, the mineral fertilizer calcium ammonium nitrate (CAN, Borealis L.A.T. GmbH, Linz, Austria) containing 27% nitrogen (1 NO_3^- / 1 NH_4^+) was applied in two conditions: First, for treatments containing biochar to balance the lack of nitrogen due to the previously described C_{tot} equality and thus to obtain the maximum fertilization of 140 kg N_{tot} ha^{-1} (MF140). And second, by adding additionally 50 kg N_{tot} ha^{-1} of nitrogen according to a common fertilization practice of local farmers (MF50) for studying the effects of organic amendments combined with mineral fertilizer. All calculations are in kilogram per hectare (kg ha^{-1}) and based on a soil depth of 30 cm with a bulk density of 1.5 t m^{-3}.

The experiment was designed to last eight weeks until the majority of *H. vulgare* plants reached their first nodal stadium (BBCH 31). The plants were cultivated in 0.5 m pipes (PVC DN 110) and the organic amendments were applied to the upper 30 cm after manually grinding them with a pestle. Each pot was sown with 4 spring barley seeds and all treatments as well as untreated controls were performed in quadruplicate. The barley cultivar Ella (*Hordeum vulgare* L. cv. Ella; DANKO Hodowla Roślin Sp. z o.o., Kościan, Poland) was selected since Surma et al. (2019) found promising grain weight per plant and grain yield per plot for this variety. In the

second week of the experiment, when plants reached their two leaf stadium (BBCH 12), two seedlings were removed from each pot and dissolved mineral nitrogen fertilizer was applied. The pots were watered twice a week to keep 60% water holding capacity. The pots were randomized and plant growth was supported by sodium-vapor lamps and a ceiling fan. The management and sampling scheme is given in Appendix M2, Figure S1.

Various biological and chemical properties were analyzed of which soil pH, soil microbial biomass, plant morphology as well as the carbon and nitrogen content within soil and plants were among the most important ones. For a more holistic evaluation, the potential microbial activity was assessed using BIOLOG EcoPlates, fluorescein diacetate assay, and a fluorometric enzyme assay and compared to the bacterial community structure which was analyzed following 16S amplicon sequencing.

2.3 Soil and Plant Sampling Procedure

2.3.1 Sampling during Conversion

For analysis of soil properties and bacterial composition during the grassland conversion (M1) soil sampling was performed using a core sampler up to a depth of 20 cm. To avoid transition effects between sub-plots, sampling was conducted in the central area of each plot (see Appendix M1, Figure S1). Therefore, 12 subsamples of each sub-plot were taken at random, directly pooled, homogenized, and sieved (2 mm). Subsequently, the samples for bacterial analyses were frozen on dry ice and stored at -80 °C. For analysis of nitrate-N, ammonia-N, total nitrogen bound (TNb), dissolved organic carbon (DON), and pH, the soil samples were stored at 4 °C. To analyze the homogeneity across the field area, soil moisture and temperature were measured using a time domain reflectometer UMP-1 BTim (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany). Sampling during conversion was performed at four sampling dates. The first sampling (IG) describes the initial status of the marginal grassland and was conducted in July 2016 (see Appendix M1, Figure S1). The second sampling (TP) describes the transitional phase during the vegetative period of *V. faba* plants and was performed in November 2016. After incorporating the leguminous plant residues, the third sampling (FS) was conducted in June 2017 to describe the final state after the conversion to arable plots. Directly adjacent to the converted plots a fourth sampling (CG) was

performed in August 2017 to describe the properties of the grassland without any management practices and therefore acts as a control. In addition, six plant samples of *V. faba* were taken from each sub-plot during the transitional phase (TP) to analyze the content of pigments (Chl *a*, Chl *b* and total carotenoids) as well as the plant's fresh weight and height.

2.3.2 Sampling of the Greenhouse Experiment

Sampling during the short-term greenhouse experiment was performed at five time points (see Appendix M2, Figure S1). The initial sampling was performed in week 1 and describes the initial state of the pots without any soil amendment and plant influence. Subsequent samplings were performed in week 2, 4 and 6 as well as finally in week 8 during harvesting describing the final state of the barley experiment. Similar to the field campaigns the soil samples were sieved (2 mm) and stored at 4, -20, and -80 °C for later chemical, enzymatic and bacterial analysis. According to the nature of pot studies, the sampled soil of the final pots consists of a mixture of bulk soil and rhizospheric soil and can thus be considered as soil-rhizosphere mixture. For measuring the extracellular enzyme activity (EEA) around 1 g of soil was taken carefully in week 2, 4, and 6 from the upper 10 cm in the center of each pot to avoid disturbance of the root development. The phenological development stage of the barley plants was determined weekly following the BBCH system according to Bleiholder et al. (2001). In addition, during the last four weeks the chlorophyll contents were measured using the non-destructive Dualex® Scientific Dx4.5 sensor (FORCE-A, Orsay, France). Furthermore, the gravimetric water content in the soil-rhizosphere mixture for each pot was determined after drying samples for 24 h at 105 ºC.

2.4 Soil and Plant Properties

2.4.1 Soil Nutrient Stocks (DOC, TNb, Nitrate-N, and Ammonium-N) and pH

For analysis of soil nutrient stocks, dissolved organic carbon (DOC), total nitrogen bound (TNb), nitrate-N (NO_3^--N), and ammonium-N (NH_4^+-N) were extracted from 5 g of field fresh samples using 20 mL of 0.01 M $CaCl_2$. Concentrations of TNb and DOC were determined in a DIMATOC®2000 device (DIMATEC, Langenhagen, Germany). NO_3^--N and NH_4^+-N were analyzed photometrically by continuous flow measurements using the autoanalyzer CFA-SAN

Plus (Skalar Analytik, Erkelenz, Germany). Measurements of the soil pH followed the guidelines of the OECD (ISO 10390, 2005) adding 25 mL of 0.01 M $CaCl_2$ to 5 g of bulk soil samples. Sub-samples of soil (M1) and soil-rhizosphere mixture (M2) were dried at 105 °C for 24 h to assess the gravimetric water content. Soil samples used for soil properties (DOC, TNb, NO_3^-, NH_4^+ and pH) were stored at 4 °C.

2.4.2 Total Carbon and Total Nitrogen in Plants

Nutrient contents (C_{tot} and N_{tot}) of *H. vulgare* plants from the greenhouse experiment (M2) were determined after drying the leaves and roots for 24 h at 60 °C. For analysis, the dried plant material was grinded using a mixer mill (MM 400, Retsch®, Haan, Germany) and subsequently analyzed in triplicates via combustion by an elemental analyzer (Euro EA, Eurovector Srl, Pavia, Italy).

2.4.3 Chlorophylls, Total Carotenoids and Leaf Area Index

Chlorophylls (Chl *a* and Chl *b*) and total carotenoids of *V. faba* plants during the conversion experiment (M1) were analyzed following the protocol of Lichtenthaler and Buschman (2001) which was slightly modified by Obermeier et al. (2015). The homogenized plant material was ground under liquid nitrogen using mortar and pestle and 0.1 g was added to 0.8 mL of cold 95 % ethanol to extract the plant pigments. Subsequently, this mixture was centrifuged at 260 g and 4 °C for two minutes. The resulting supernatant was collected in Eppendorf tubes and the pellet was again added to 0.8 mL 95 % ethanol, stirred, and centrifuged. Subsequently, the supernatant was added to the first extract. This procedure was repeated three times and 1 mL of the extract was used for the spectrophotometric analyses of the pigment contents in *V. faba* plants (M1). Absorption of each sample was recorded at specific wavelengths of 664.1, 648.6, and 470 nm, using a DU®800 Series UV-Vis spectrophotometer (Beckman Coulter Inc., Webster, United States). The pigment contents are given in mg g^{-1} *fw* (Lichtenthaler and Buschman 2001) and all measurements were performed in triplicates. Images of scanned shoot leaves from the final sampling of the greenhouse experiment (M2) were taken using the Epson Perfection 4180 Photoscanner and analyzed for their green pixel content to obtain the leaf area index (LAI) with MATLAB (The MathWorks Inc., Natick, USA).

2.5 Soil Microbial Analyses

2.5.1 Microbial Biomass and Potential Soil Enzyme Activity

During the greenhouse experiment (M2), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined following chloroform fumigation of 5 g fresh soil with ethanol-free chloroform in a desiccator for 24 h. The subsequent extraction and analysis of DOC and DON were performed as described above for soil nutrient stocks using the DIMATOC device (see 2.4.1). MBC and MBN were calculated by subtracting the non-fumigated fraction (DOC and DON) and dividing the results by using the factors k_{EC} (0.45) and k_{EN} (0.54), respectively (Brookes et al., 1985; Vance et al., 1987).

For determination of the potential soil enzyme activities of soil samples from the greenhouse experiment (M2) three different soil enzyme assays were performed.

The **overall potential microbial activity** was assessed following the hydrolysis of fluorescein diacetate in a commercial assay (3',6'-diacetylfluorescein) (FDA) and reflects the activity of different secreted soil enzymes, such as proteases, esterases and lipases (Green et al., 2006). In short, 1 g of soil was mixed with 50 mL of 60 mM sodium phosphate buffer (pH 7.6) and 0.5 mL 4.9 mM FDA-acetone. After incubating the suspension for 3 h and 37 °C in the dark and terminating the reaction with 2 mL acetone a supernatant was obtained following centrifugation for 5 min at 6000 rpm. The supernatant was transferred to 96-well plates in triplicates and the absorbance of the remaining FDA was measured at a wavelength of 490 nm using a FLUOstar® Omega Plate Reader (BMG Labtech, Ortenberg, Germany). The overall potential microbial activity was determined using a standard curve measured at 490 nm with 2, 5, 8, 11 and 15 µg mL^{-1} fluorescein and is given in mg fluorescein kg^{-1} *dw* h^{-1}.

The **carbon metabolization rate** of soil microorganisms was assessed using BIOLOG EcoPlates™ (Biolog Inc., Hayward, United States) reflecting the utilization of 31 different carbon sources. For this, 1 g of soil was suspended in 10 mL of 10 mM phosphate-buffered saline (PBS) (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.4) mixed for 20 min at room temperature and incubated for 30 min at 4 °C. For reduction of cell counts, 1 mL of the suspension was diluted with 20 mL PBS and subsequently 130 µL of the resulting supernatant was transferred to EcoPlates and incubated for six days at 30 °C. Absorbance was measured

at 595 nm after 0, 3, 6, 21, 24, 48, 72 and 144 h using a FLUOstar® Omega Plate Reader. After blank correction (Li et al., 2018) and standardizing the absorbance, the net area under the absorbance curve (AAT) was calculated according to Guckert et al. (1996). The mean of the 31 AAT values is given as average well color development (AWCD).

The **potential extracellular enzyme activities** (EEA) in the soil-rhizosphere mixture were measured using the fluorescent dye 4-methylumbelliferone (MU; Sigma-Aldrich, St. Louis, United States) according to Pritsch et al. (2005). For analysis of different extracellular enzymes, which provide important information about organic matter decomposition and nutrient cycling (Jackson et al., 2013), three different MU-labeled substrates were used. In short, 0.4 g of the soil mixtures were mixed for 15 min in 40 mL Milli-Q water, homogenized in an icy ultrasonic bath for 3 min, and subsequently filtered with Miracloth paper (pore size 22-25 µm; VWR™, Darmstadt, Germany). Fifty µl of the filtered soil suspension were incubated in triplicates in opaque 96-well plates (VWR™, Darmstadt, Germany) at 20 °C with 100 µl of the respective substrate saturation solution (Pritsch et al., 2004). Appropriate substrate concentrations and incubation times were determined in a pre-experiment for each of the respective substrate/corresponding enzyme combinations: MU-*β*-D-glucopyranoside/*β*-glucosidase (MUG, EC 3.2.1.21) 600 µM and 60 min, MU-N-acetyl-*β*-D-glucosaminide/*β*-N-acetylhexosaminidase (MUN, EC 3.2.1.52) 100 µM and 60 min and MU-phosphate/acid phosphatase (MUP, EC 3.1.3.2) 600 µM and 40 min. Enzymatic reactions were terminated by adding 100 µl of 1.25 M TRIS buffer (pH > 10) before the plate was centrifuged for 3 min at 2420 rpm. 20 min after termination the fluorescence was measured at wavelengths of 365 nm for excitation and 450 nm for emission using a SpectraMax® Gemini™ EM microplate reader (Molecular Devices, Ismaning, Germany). For each run a MU calibration curve (0, 1, 2, 3, 4, 5, 6 and 7 µM MU in Milli-Q water) and a soil quenching control (4 µM MU in soil suspension) were performed. The maximum activity was expressed in picomol MU per gram dry soil per hour (pmol MU g^{-1} *dw* h^{-1}) according to German et al. (2011).

2.5.2 Nucleic Acid Extraction

For analysis of the bacterial community structure, the DNA of bulk soil and the soil-rhizosphere mixtures were extracted from 0.5 g soil samples using the Fast DNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, United States) according the manufacturer's protocol and subsequently

stored at -80 °C. Slight modifications comprised a DNA homogenization for 30 s at 5500 rpm using a FastPrep homogenizer (MP Biomedicals, Eschwege, Germany) and a DNA elution from the binding matrix using 50 µL DES elution solution with 5 min incubation time at room temperature. Negative controls were included using empty extraction tubes. DNA concentrations were quantified in duplicates using the Quant-iTPico™ Green® ds DNA assay Kit (Thermo Fisher Scientific, Waltham, United States) following the manufacturer's protocol. Measurements were performed at 520 nm using the SpectraMax Gemini EM Fluorescence Plate Reader Spectrometer (Molecular Devices, Ismaning, Germany) and the Qubit™ 4 Fluorometer (Thermo Fisher Scientific, Waltham, United States). Non-target controls (NTC) were used to correct for background fluorescence. DNA purity and quality was assessed calculating the absorbance ratios for wavelengths 260nm/280nm and 260nm/230nm measured with a NanoDrop™ 1000 spectrometer (PeQlab Biotechnoloy, Erlangen, Germany). All DNA extracts were stored at -80 °C for further usage.

2.5.3 16S Library Preparation and Illumina Sequencing

For the preparation of soil and soil-rhizosphere samples for Illumina sequencing, 1 ng of the previously described DNA extracts were used to amplify the hypervariable V1-V2 region of the 16S rRNA gene via Polymerase chain reaction (PCR). The forward primer S-D-Bact-0008-a-S-16 (5′-AGAGTTTGATCMTGGC-3′) and the reverse primer S-D-Bact-0343-a-A-15 (5'-CTGCTGCCTYCCGTA-3') were used (Klindworth et al., 2013). All amplifications were performed in triplicates and PCR conditions were: initial denaturation at 98 °C for 30 s, followed by 28 cycles each at 98 °C for 10 s (denaturation), 60 °C for 30 s (annealing) and 72 °C for 30 s (elongation), followed by 72°C for 5 min (final elongation). A non-target control (NTC) and a positive control containing the target gene were also performed with the same PCR conditions. The PCR reaction mix contained 12.5 µL of NEBNext High-Fidelity Master Mix (New England Biolabs, Ipswich, United States), 5 pmol of each primer, 10.5 µl of molecular grade water (DEPC-treated), 1 ng of DNA extract and 2.5 µL of 3 % bovine serum albumin (BSA) to enhance the amplification yield. Quality of PCR amplicons was checked by gel electrophoresis, loading 5 µL of the PCR product on a 1 % TRIS-acetate-EDTA (TAE) agarose gel containing ethidium bromide. Subsequently, triplicate PCR reactions were pooled and purified using Agencourt® AMPure®XP kit (Beckman Coulter Inc., Webster, United States) following the manufacturer's

instructions for 96-well plates (modified by 78 µL beads for 60 µL of PCR product). Quantity and quality of purified DNA and its controls were controlled on a Fragment Analyzer™ (Advanced Analytical Technologies GmbH, Heidelberg, Germany) using the DNF-473 Standard Sensitivity NGS Fragment Analysis Kit (1 bp – 6000 bp). Subsequently, an indexing PCR was performed for facilitating pooling and thus the simultaneous sequencing of the library. Therefore, the Nextera XT Index Kit v2 (Illumina Inc., San Diego, United States) was used to index 10 ng of the 16S rRNA gene amplicons, according the manufacturer's protocol. The Indexing-PCR comprised initial denaturation with 98 °C for 30 s, followed by 8 cycles each at 98 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s, ending with a final elongation at 72 °C for 5 min. Indexed PCR products were purified, and quality as well as quantity were checked as described above. All amplicons were diluted to 4 nM using DEPC-water for further equimolar pooling. Finally, 10 pM of the indexed DNA pool was used for next-generation sequencing on an Illumina MiSeq platform (Illumina Inc., San Diego, United States) using the MiSeq Reagent Kit v3 (600 cycle) for paired-end sequencing. As positive control, PhiX (Illumina Inc., San Diego, United States) was used as a spike-in.

2.5.4 Sequencing Data Processing

For processing the demultiplexed raw data received from the Illumina Sequencing platform, the software AdapterRemoval (V. 2.1.7) was used to remove primers and adapters separately for reverse and forward reads (Lindgreen, 2012). Subsequently, correcting of sequenced amplicon errors followed the model-based approach of the R package DADA2 (V. 1.8.0) (Callahan et al., 2016). After checking ten read quality plots separately for forward and reverse reads, quality filtering was performed with a maximum expected error of three and a minimum read quality of two. For the conversion experiment (M1) trimming of forward reads was performed at 10 and 200 bp and reverse reads at 60 and 180 bp only. According to differences in the quality plots, trimming of sequences obtained from the greenhouse experiment (M2) was performed at 10 and 250 bp for forward and 10 and 200 bp for reverse reads. Remaining contaminations of PhiX sequences were removed during quality filtering. Subsequently, error modelling of the reads was performed using the DADA2 algorithm for denoising the trimmed sequence data and chimeras had been removed. To obtain the final denoised sequence table the forward and reverse reads were merged and an amplicon sequence variant (ASV) table was constructed. Finally, taxonomy was

assigned to the chimera-free ASV table using the naive Bayesian classifier method (Wang et al., 2007) against the SILVA database (V. 128) (Quast et al., 2013).

The sequence data were imported to R (V. 3.5.1) (R Core Team, 2018) using the phyloseq package (V. 1.25.2) (McMurdie and Holmes, 2013). To analyze the sequence depths for each sample rarefaction curves were calculated using the vegan package (V. 2.5-4) (Oksanen et al., 2018). Subsequently, the sequence data was filtered by removal of ASVs that were not assigned to bacteria and archea (NA and eukaryota) as well as ASVs assigned to chloroplasts and mitochondria. In addition, ASVs present in either negative controls or only a single sample were removed. Due to wide variations observed for sequence depths of samples from the greenhouse experiment (M2), the filtered sequences were subsampled to the lowest read count over all samples with the 'rarefy'-function (vegan package).

2.6 Statistical Analysis

All soil, plant and microbial data were statistically analyzed using R (V. 3.5.1) (R Core Team, 2018). A one-way independent ANOVA ($p < 0.05$) was performed using basic R-functions for normal distributed data and Kruskal-Wallis test for not normal distributed data. Differences in soil, plant and microbial data were confirmed in conjunction with Tukey's post-hoc test using the package agricolae V. 1.3.0 (De Mendiburu, 2014). Plotting was performed using the ggplot2 package (V. 3.0.0) (Wickham, 2016). For further microbial analysis of the greenhouse experiment (M2) the term variant was established to separate the initial soil from the pool of the fourteen treatments, including the control. Normality of data was assessed by Shapiro-Wilk test and homogeneity of variance within each group by Bartlett test. Pearson and Spearman rank correlation test for normal and not normal distributed data, respectively was used to find shared variation between the measurements and to show correlations between soil, plant and microbial data of the greenhouse experiment (M2). To express the shared variation, the coefficient of determination (r^2) was calculated and presented in percentage (Field et al., 2012). Relative abundances of microbial data and standard deviation are shown on phylum, order, family, and genus level to indicate effects of the management practices (M1), fertilizer combinations (M2) as well as the homogeneity within the replicates of both experiments (M1 and

M2). Tukey's post-hoc test in conjunction with a one-way ANOVA was run for relative abundances to highlight which sampling time point (M1), and fertilizer treatment (M2) differs significantly from others ($p < 0.05$). More detailed statistics of the filtered sequence data were performed in R using the phyloseq package (V. 1.25.2). Bacterial α-diversity was calculated using the 'plot_richness'-function of the phyloseq package. To reveal differences of the bacterial composition during the grassland conversion (M1), bacterial richness and Simpson's diversity index are shown. Shannon diversity index and Pielou's evenness index are given to express variations induced by different fertilizer combinations within the soil-rhizosphere mixture of the greenhouse experiment (M2). Changes between the sampling points during the grassland conversion (M1) were furthermore visualized via non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities on genus level. NMDS was done for a reduction of two dimensions with a maximum of 500 tries using the vegan package. Ninety-five percent confidence ellipses were plotted for each sampling time to show the trend of the bacterial *β*-diversity. Bacterial *β*-diversity of the greenhouse experiment (M2) also based on Bray-Curtis dissimilarities and was visualized by ordination using the multivariate principal coordinate analysis (PCoA) approach. To confirm the PCoA results a permutational multivariate analysis of variance (PERMANOVA) was performed using the vegan package. Associative relationships of plant and soil parameter to the most abundant bacterial families found during the greenhouse experiment (M2) were identified using multivariate ANOVA ($p < 0.01$). The nucleotide sequence data are available in the NCBI Sequence Read Archive (SRA) (Leinonen et al., 2011) under the BioProject accession numbers given in Table 2.

Table 2 Bioproject accession number (BioProject ID) and corresponding hyperlink (Link) to access the raw data of the conversion and greenhouse study (Experiment) examined within this thesis.

Experiment		**BioProject ID**	**Link**
Conversion	M1	PRJNA471669	https://www.ncbi.nlm.nih.gov/sra/PRJNA471669
Greenhouse	M2	PRJNA540756	https://www.ncbi.nlm.nih.gov/sra/PRJNA540756

3 Manuscript Overview

The following section summarizes the three manuscripts that are embedded in this thesis, their publication status and highlights the contribution of the authors.

I. Manuscript I (M1, first author, published)

Obermeier MM, Gnädinger F, Durai Raj AC, Obermeier WA, Schmid CAO, Balàzs H, Schröder P, 2020. Under temperate climate, the conversion of grassland to arable land affects soil nutrient stocks and bacteria in a short-term. Science of the Total Environment, 703, 135494

II. Manuscript II (M2, co-first author, published)

Obermeier MM, Minarsch Eva-Maria, Durai Raj AC, Rineau F, Schröder P, 2020. Changes of soil-rhizosphere microbiota after organic amendment application in a *Hordeum vulgare* L. short-term greenhouse experiment. Plant and Soil, 455, 489-506

III. Manuscript III (M3, co-author, published)

Schröder P, Beckers B, Daniels S, Gnädinger F, Maestri E, Marmiroli N, Mench M, Millan R, Obermeier MM, Oustriere N, Persson T, Poschenrieder C, Rineau F, Rutkowska B, Schmid T, Szulc W, Witters N, Sæbø A, 2018. Intensify production, transform biomass to energy and novel goods and protect soils in Europe - A vision how to mobilize marginal lands. Science of the Total Environment, 616, 1101-1123

Manuscript I (M1) - Under temperate climate, the conversion of grassland to arable land affects soil nutrient stocks and bacteria in a short-term

Obermeier MM, Gnädinger F, Durai Raj AC, Obermeier WA, Schmid CA, Balàzs H, Schröder P

Published in Science of the Total Environment (2020), Volume 703,
DOI: 10.1016/j.scitotenv.2019.135494

Manuscript (M1) highlights short-term effects on soil nutrient stocks and soil functions after the conversion of a former extensively used marginal grassland to arable land.

The effects of grassland removal, tillage, intercropping with faba bean (*Vicia faba* L.) and its later incorporation were studied with focus on soil properties and bacterial composition. Composite samples were collected from the topsoil (0–20 cm) in (a) the initial grassland, (b) the transitional phase during growth of *V. faba*, (c) after ploughing the legume in, and (d) untreated controls. Nitrate-N, ammonium-N, dissolved organic carbon (DOC) and total nitrogen bound (TNb) were analyzed and comparisons of the bacterial composition after 16S-amplicon sequencing were performed to assess soil functions. Mineralization after grassland conversion followed by the biological nitrogen fixation of broad beans enhanced the nitrate-N content in bulk soil from 4 to almost 50 $\mu g\ N\ g^{-1}$ *dw*. The bacterial community structure on phylum level in bulk soil was dominated by *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes* and remained almost stable. However, α- and β-diversity analysis revealed a change of the bacterial composition at the final state of the conversion. This change was primarily driven by increasing abundances of the genera *Massilia* and *Lysobacter*, both members of *Proteobacteria*, after the decay of the leguminous plant residues and were found to be beneficial for soil health and plant performance. Furthermore, increasing abundances of the family *Gaiellaceae* and its genus *Gaiella* fostered this change and were related to the decreasing carbon to nitrogen ratio. To sum up, the applied management strategy was found to be suitable to replace mineral fertilizer aiming to sustainably intensify agricultural production even on converted grassland.

Contributions (all authors contributed to the comments given in the review process):
Idea for the manuscript: Obermeier MM, Schröder P
Field preparation, sampling, lab work: Obermeier MM, Gnädinger F, Balàzs H
Data analysis: Obermeier MM, Durai Raj AC, Obermeier WA, Schmid CAO
Manuscript draft: Obermeier MM

Manuscript II (M2) - Changes of soil-rhizosphere microbiota after organic amendment application in a *Hordeum vulgare* L. short-term greenhouse experiment

Obermeier MM, Minarsch Eva-Maria, Durai Raj AC, Rineau F, Schröder P

Published in Plant and Soil (2020), Volume 455, pp 489-506
DOI: 10.1007/s11104-020-04637-7

Manuscript (M2) demonstrates effects after the application of different fertilizer combinations on soil quality and plant growth in a short-term greenhouse experiment.

Addressing the constant decrease of soil quality from arable land, we performed mechanistic studies with focus on effects of organic amendments combined with mineral fertilizer on soil-rhizosphere microbiota and its influence on soil quality and plant performance. Therefore, a short-term greenhouse experiment was conducted with pelletized spent mushroom substrate, blended with digestate and straw, and various amounts of biochar and mineral fertilizer, to investigate effects on agricultural soil and performance of *Hordeum vulgare* L. Different biological and chemical properties, microbial activity, bacterial diversity and plant performance were assessed to evaluate soil quality. Plant performance was intensified across all fertilizer combinations. Bacterial β-diversity revealed the most pronounced variation between the initial and final sampling, indicating a strong influence of plant development on the soil-rhizosphere mixture. Microbial activity (FDA), potential enzyme activity and the metabolic diversity of microbial communities (BIOLOG) were not affected by the different amendments, whereas changes of the bacterial composition on family level were observed, indicating functional redundancy. The treatment containing biochar and the highest rate of mineral fertilizer caused the strongest changes compared to other treatments and controls. Conclusively, we found organic amendments, in particular the pellets used, to be suitable for improved plant performance and maintained soil health and thus recommend its application to replace the input of mineral fertilizer on arable fields for a more sustainable crop production.

Contributions (all authors contributed to the comments given in the review process):
Idea for the manuscript: Obermeier MM, Minarsch EM, Schröder P
Greenhouse preparation, sampling, lab work: Obermeier MM, Minarsch EM, Rineau F
Data analysis: Obermeier MM, Minarsch EM, Durai Raj AC
Manuscript draft: Obermeier MM, Minarsch EM

Manuscript III (M3) - Intensify production, transform biomass to energy and novel goods and protect soils in Europe - A vision how to mobilize marginal lands

Schröder P, Beckers B, Daniels S, Gnädinger F, Maestri E, Marmiroli N, Mench M, Millan R, Obermeier MM, Oustriere N, Persson T, Poschenrieder C, Rineau F, Rutkowska B, Schmid T, Szulc W, Witters N, Sæbø A

Published in Science of the Total Environment (2018), Volume 616-617, pp 1101-1123
DOI: 10.1016/j.scitotenv.2017.10.209

The opinion paper (M3) sketches challenges of a smart agricultural intensification and recent developments to restore marginal land while also protecting soils across Europe.

We elucidated aspects to counteract the enduring degradation of fertile soils aiming to convert marginal land into economically attractive arable land while also maintaining or even restoring valuable soil ecosystem services. Different scenarios of the interdisciplinary project (INTENSE) are shown and a toolbox is given to help decision makers to optimize the transformation of marginal land into productive land. Besides the importance of an initial detection of the individual weaknesses of a given site the role of various soil amendments to increase long-term productivity (e.g. compost, municipal slurries, manure, digestates, and biochar) are discussed and the opportunity to lower fertilizer input with improved nutrient use efficiency (NUE) when choosing the right source, right rate, right time and right place of amendment application. To support decision making and farm management precise tools like proximal sensors or drones equipped with multispectral cameras will be essential. In addition, general mechanisms of the plant-microbe interaction (e.g. nutrient cycling, nutrient availability, biosynthesis of phytohormones) are explained and their influence on soil quality, plant health and plant performance. Finally, indicators and models are discussed and the importance of an economic valuation of biodiversity and selected management practices to involve all stakeholders (e.g. farmers, policy makers, and consumers) to establish economically sound management systems to unlock the potential of marginal land. We conclude, that the challenge is no longer simply to maximize productivity of a single crop, but to optimize farming across a far more complex landscape of production, environment, and social outcomes.

Contributions (all authors contributed to the comments given in the review process):
Idea for the manuscript: Schröder P
All authors contributed to the manuscript draft and wrote different chapters

4 General Discussion

This thesis, embedded in the INTENSE project of the FACCE-JPI funded by the EU, aims to contribute to an intensified agricultural production by restoring marginal land and using organic amendments while also protecting soils in Europe (M3). In order to face the enduring decline of fertile soils two main questions arose which were addressed within our experiments, first whether it is possible to convert marginal grassland to arable land gently and in a sustainable manner to unlock its potential for future crop cultivation (M1). And second, whether organic amendments alone or in combination with mineral fertilizer are suitable to improve plant performance while at the same time protecting soil functions, aiming to reduce the input of mineral fertilizer on arable fields (M2). Therefore, we initially converted a marginal grassland from which, according to the farmer, no income could be generated, into fertile arable land through the intermediate cropping of *V. faba* (M1). Thereafter, we investigated effects after applying various compositions of organic amendments and mineral fertilizer during *H. vulgare* cultivation on plant performance, nutrient stocks and microbial mechanisms in a holistic approach (M2). The focus was on soil amendments (e.g. pellets, biochar) which were derived after transforming biomass residues (e.g. spent mushroom substrates, chopped pruning residues) aiming to contribute to close production circles in a sustainable bioeconomy (M3). The main steps of the grassland conversion (Initial grassland, Transitional phase, Final state) and the fertilizer experiment (Amendments, Plant growth) as well as the main drivers on shaping the nutrient stocks and bacterial composition in soil are sketched in Figure 1.

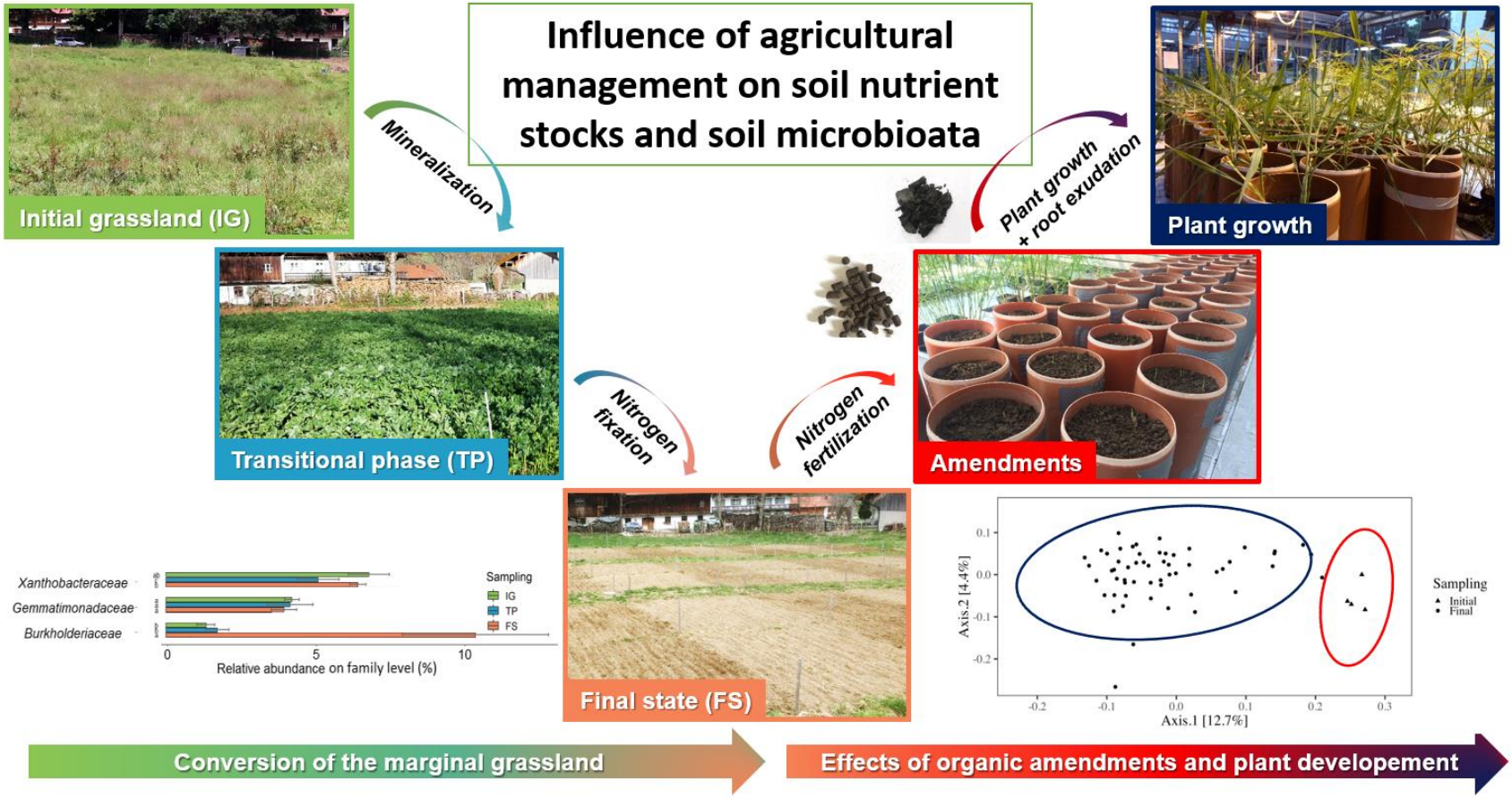

Figure 1 Scheme to illustrate the different experiments to study the influence of agricultural management practices on soil nutrient stocks and soil microbiota. The initial steps (Initial grassland, Transitional phase, and Final state) are part of the conversion experiment which aimed at unlocking the potential of the marginal grassland. The effects of different combinations of organic amendments and mineral fertilizer on *H. vulgare* plants are shown on the right side of the illustration (Amendments and Plant growth) highlighting the strong influence of nitrogen fertilization and plant growth and associated mechanisms on shaping soil microbiota.

4.1 Unlocking the Potential of Marginal Land

4.1.1 Necessity for Land Conversion and Global Evaluation

Due to the globally increasing food demand mentioned in the beginning, the increasing sealing of arable soil, the additional cultivation of fodder and raw materials for bioenergy production (Rathmann et al., 2010) and multiple other factors the pressure on fertile arable soils is going to exacerbate in the near future. In consequence it will be necessary to combine and optimize novel and traditional agricultural management approaches to provide enough food, feed and biomass for the increasing world population while alleviating its impact on the environment and global climate. Negative impacts of intense agricultural management on atmosphere (e.g. greenhouse gas emissions), lithosphere (e.g. soil acidification), hydrosphere (e.g. groundwater contamination), and biosphere (e.g. loss of biodiversity) have already been observed (Cameron et al., 2013; Horrigan et al., 2002; Tsiafouli et al., 2015). Therefore, it will be inevitable to overcome the conventional approach of intense agriculture which is primarily based on a tremendous input of synthetic nitrogen fertilizer and other agrochemical compounds on arable fields (Carvalho et al., 2017). Since this is likely to cause a lower agricultural productivity which in turn demands larger areas for maintaining agricultural production it will be essential to reconvert previously set-aside land for crop and biomass cultivation. In this context, it is important to assess and differentiate the individual weaknesses and strengths of given sites to align them to their specific suitability and to optimize their future management (M3). For example, are polluted or less productive soils more suitable for the cultivation of biomass for energy production while healthy and fertile soils should have the primacy for the cultivation of nutritious food and fodder for humans and animals?

In order to re-establish neglected or marginal land into economically attractive and fertile cropland even the gentle conversion of grassland plots may be considered if it can be achieved sustainably. Although grassland provides important ecosystem services and is recognized for its high biodiversity (Bengtsson et al., 2019) it might be more sustainable in a global perspective to replace, for instance, the tremendous import of soybeans grown on fields originated from deforested tropical rainforests (slash-and-burn agriculture) with domestic crop production. Since tropical rainforests exert important ecosystem services such as carbon sequestration, maintaining water supply, influencing temperature and precipitation (Pedrinho et al., 2019) and further act as

potential N_2O sink (Merloti et al., 2019) their destruction influences global climate, freshwater, biodiversity, food and even human health more than any other terrestrial biome around the globe (Brandon, 2014). Thus, European soybean imports cause losses of important ecosystem services without beneficial societal or socio-economic effects on a global scale (Boerema et al., 2016) and the transportation around the globe additionally enlarges its ecological footprint (Gil, 2020). Interestingly, even decreases in permanent meadows and pastures in importing countries due to the substitution of grass as feed have been reported since soybeans are primarily imported as animal feedstock (Boerema et al., 2016). To stop or at least level the import of food and feed and to alleviate its concomitant thread on the global environment we surveyed how the conversion of a marginal grassland under temperate climate to arable land in southern Bavaria could yield biomass or fodder. The study aimed at revealing responses on soil nutrient stocks and soil bacteria caused by breaking-up the grass scar and the transitional leguminous nitrogen fixing phase and to provide high-quality crop land (M1).

4.1.2 Effects of the Conversion Strategy on Soil Nutrient Stocks

The initial site was identified as grassland with moderate quality, slightly moist and without indication of salt stress. Thus it was found to be suitable to be converted for agricultural crop production. Already in the beginning of the conversion, after milling and ploughing the initial grassland and during *V. faba* growth, our study revealed a strong three-fold increase of NO_3^- and TNb compared to the initial situation. This increase highlights the great potential of mineralization processes to mobilize the soil nitrogen pool after breaking-up the grass scar and incorporating the residual green. Similar effects for soil nitrogen dynamics had been observed by Chen et al. (2014) who reported a strong mineralization after plant residue incorporation. Subsequently, the accumulation of NO_3^- and TNb was followed up by another three-fold increase towards the end of the conversion which finally led to 50 µg NO_3^--N g^{-1} *dw* equally to 150 kg N ha^{-1}. This increase was dominated by the capability of the legume *V. faba* to biologically fix atmospheric nitrogen. Its subsequent incorporation into the soil reveals the benefits of leguminous intercropping for plant nutrition (Fan et al., 2006; Ordóñez-Fernández et al., 2018). The enrichment of NO_3^- in the final state of the experiment seemed to be ideal for further cropping on arable land and clearly demonstrates how a sustainable conversion strategy can contribute to unlock the potential of a formerly neglected marginal grassland. It further highlights the possibility of influencing the

natural nitrogen transforming flows and introducing huge amounts of plant available nitrogen into soil with the application of green manure and without introducing any additional organic or inorganic fertilizer produced outside the farm (M1). Crews and Peoples (2004) reported that obtaining nitrogen from legumes is more sustainable than from industrial processes.

In contrast to the strong increase of NO_3^- and TNb, the amount of dissolved organic carbon (DOC) reached its maximum during growth of *V. faba* and subsequently decreased in the end of the experiment. The decrease of DOC following the incorporation of the leguminous crop residues might be explained by the utilization of soil organic carbon for microbial nitrogen immobilization (Reichel et al., 2018) and lead to a decreasing DOC/TNb ratio throughout the conversion.

4.1.3 Microbial Responses during Grassland Conversion

Although the observed enrichment of NO_3^- tempts to assume that the preparation of the arable field might already be successful, multiple parameters need to be considered to evaluate whether such a conversion is sustainable in a bigger perspective. Since modern molecular approaches are enabling the consideration of the microbial composition at affordable costs, and the awareness of the importance of microbial mechanisms e.g. on shaping nutrient cycles and thus influencing soil health and plant performance raises, a microbial approach might be the perspective of choice for in-depth analysis of changes within the converted soil. In this respect our study aimed at revealing microbial responses induced by the different conversion steps to evaluate its sustainability as case study for the restoration of marginal sites.

Interestingly, both, the bacterial composition on phylum level and the bacterial richness were found to remain quite stable during the conversion (M1), which indicates the resilience of the grassland soil and furthermore is a first indication of a gentle transformation strategy. Most prominent phyla during the complete conversion were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes* which are common for temperate grassland soils (Delgado-Baquerizo et al., 2018; Kaiser et al., 2016). The only impact on phylum level was observed for members of the predominant phyla *Proteobacteria* after incorporating the leguminous plant residues. Since *Proteobacteria* are well known indicators for crop residue degradability (Pascault et al., 2010) higher occurrence during decomposition of *V. faba* might be obvious. In more detail, this increase was primarily driven by the very strong increase of the family

Burkholderiaceae and herein especially by the genus *Massilia* that was found to dominate the final plots after the conversion (M1). Members of the genus *Massilia* are known to be plant growth-promoting rhizobacteria in leguminous plants and are associated to the plant's endo- and rhizosphere (Ofek et al., 2012; Xiao et al., 2012). This explains their strong enrichment at the final state of the conversion, since after the bacterial decomposition of leguminous plant material the bacteria will be released to the soil. Similarly, Pascault et al., (2010) observed such a strong increase of *Massilia* especially in early stages of plant decomposition. Interestingly, members of the genus *Massilia* have been described to produce siderophores, phytohormones (e.g. IAA) (Ofek et al., 2012) and N-Acyl homoserine lactones (AHLs) which are known to stimulate plant growth and even to induce systemic resistance against pathogens (D'Angelo-Picard et al., 2005; Schuhegger et al., 2006). Consequently, some members of the genus *Massilia* might be promising candidates as bacterial inoculant for improved sustainable management practices and can indicate soil health. Furthermore, members of the genus *Massilia* are known to be NO_3^- and NO_2^- reducers which might additionally explain their high abundance at the end of the experiment when NO_3^- was highest (Zhang et al., 2006; Bailey et al., 2014) and furthermore highlights their beneficial role to balance the nutrient pool in healthy soil.

Similarly, members of the second most abundant genus, *Gaiella (Actinobacteria)*, were found in highest occurrence at the final state of the conversion. Interestingly, Hermans et al. (2017) reported a negative correlation of the abundance of its superordinate family *Gaiellaceae* to the C/N ratio in soil, similar to our findings with its highest abundance in the final plots when DOC/TNb ratio was lowest. These authors highlight the role of this family as good biological indicator for the carbon to nitrogen ratio in soil.

On order level, all grassland plots were dominated by *Rhizobiales* (*Proteobacteria*) which are common within temperate grassland soil (Kaiser et al., 2016). Decreasing abundances during growth of *V. faba* were associated to lower plant density and species diversity following the grass scar removal. Subsequent increases at the final state of the conversion may be explained by decomposition of leguminous plant residues comparable to the mechanisms observed for *Massilia*. Effects of rhizobia escaping from senescing nodules have already been reported (Denison and Kiers, 2011) and emphasize once more the influence of decomposition processes on shaping the bacterial composition in soil. It furthermore points to the success of the management strategy,

since although the abundance of the predominant order (*Rhizobiales*) slightly decreased during growth of *V. faba* its abundance recovered due to the later incorporation of plant biomass and associated mechanisms. This might be beneficial even for later crop cultivation since rhizobia are able to repopulate the soil, survive in the soil between different hosts before infecting new hosts for symbiosis (Denison and Kiers, 2011) where they exert their plant growth promoting potential (Glick, 2012). Some rhizobia are found to survive in soil for months and even years and it was reported that root exudates, even from nonhosts, are likely to support their survival and reproduction (Denison and Kiers, 2011). Their ubiquitous and high occurrence in many soils, the fast response to soil changes (e.g. tillage) and their association to biological nitrogen fixation make rhizobia furthermore a good indicator for soil quality (Torabian et al., 2019).

Another important finding during the grassland conversion (M1) is the strong increase of the genus *Lysobacter* (*Proteobacteria*) after incorporating leguminous crop residues. This has already been shown for tillage-residue managements systems (Chávez-Romero et al., 2016). Members of this genus have been described as promising candidates for the biological control of plant diseases (Hayward et al., 2010) and their enriched occurrence might also be a good indicator for improved soil quality (Wang et al., 2017). Together with the strong increase of *Massilia* (*Proteobacteria*) and the recovery of *Rhizobiales* (*Proteobacteria*) this is a very promising finding. It shows that the changes observed within the bacterial composition at the final state of the conversion (M1) were dominated by increasing occurrence of bacteria (*Massilia, Lysobacter, Rhizobiales*) which are described to be beneficial for soil health, plant growth and plant disease protection. Since at the same time the soil nutrient pool (e.g. NO_3^-) increases, the abundance of these microbes indicates the success of the sustainable conversion strategy.

The latter observation is furthermore of importance since the highly diverse bacterial community structure, as pointed out by Simpson's index of diversity, became smaller towards the end of the conversion (M1) indicating a shift to more dominant species when plant cover was lacking. Since normally such a decrease of diversity would indicate a disturbance of important soil functions (Tsiafouli et al., 2014; Wall et al., 2015) this is of special interest because with the given soil under the given conditions the bacterial composition is resilient enough to provide important soil functions although a disturbance slightly altered its composition. Since also the β-diversity on genus level revealed the most pronounced change of the bacterial composition at the final state of

the conversion (M1) the shift to higher occurrence of beneficial microbes is a good and important indicator for the great potential of such a gentle conversion strategy to recover marginal soils for food, feed and biomass production.

4.1.4 Additional Parameter and Final Evaluation

The constant soil pH during the grassland conversion (M1) furthermore supports our finding that it is possible to gently transform grassland since the prevailing range of pH 5.4 ± 0.2 was found to be optimal for root nodule formation in *V. faba* (about 60 per plant) (Torabian et al., 2019). This is an important advantage of leguminous nitrogen fixation since the alternative introduction of e.g. ammonium-based fertilizers, to enrich the soil nitrogen pool, is known to acidify soils with negative impact on crops, microbes and important belowground soil functions (Crews and Peoples, 2004; Hao et al., 2020). Soil acidification at the given pH would in particular cause major nutrient deficiencies (e.g. nitrogen, phosphorus, potassium, calcium, magnesium) (Fernández and Hoeft, 2009), reduce soil respiration, fine root biomass as well as MBC and MBN (Meng et al., 2019) and thus might even cancel the positive effects on soil nutrition as shown for acidic Ultisols in southern China (Liu et al., 2018a). Furthermore, soil acidification, due to continuous nitrogen enrichment and sulfur deposition, has become a global environmental issue (Meng et al., 2019) that needs to be addressed when management strategies embedded in a more sustainable agriculture are developed. It can furthermore be alleviated with optimizing the fertilizer composition, the nitrogen rate and the return of straw (Hao et al., 2020). Since pH is also considered to be one of the most important drivers of bacterial community structures in arable and grassland soils (Kaiser et al., 2016, Liu et al., 2018b; Wu et al., 2017), it is furthermore of special concern for maintaining soil health. During grassland conversion however (M1), instead of pH the main drivers for shaping the soil bacterial composition have been found to be the effects involved in the decomposition processes, the strongly increasing NO_3^- content and the decreasing DOC/TNb ratio.

Overall, the conversion study (M1) substantiates the successful transformation of former neglected and marginal grassland of moderate quality into economically attractive and fertile arable land. The strong enrichment of plant available NO_3^- during the conversion (M1) has shown the great potential of mineralization processes after breaking-up grassland together with leguminous nitrogen fixation to restore marginal land for future crop cultivation. The incorporation of grass

and leguminous plant residues are basically the application of green manure which is one option for more sustainable cropping systems aiming to reduce the global requirements of synthetic mineral fertilizer while improving soil physical, chemical and biological properties (Fageria, 2007). Even beneficial effects on soil microbial composition have been observed (e.g. *Massilia, Lysobacter, Rhizobiales*). However, such gentle conversion of grassland should be carried out just in exceptional conditions since grassland itself provides important ecosystem services like maintaining water supply and retention, storing carbon, preventing erosion, mitigating climate change, improving pollination and even social and cultural values (Bengtsson et al., 2019) which were only partly balanced with the intermediate cultivation of *V. faba* and the provision of arable land. Nevertheless, it can be assumed that it might be more sustainable in a global perspective to convert agricultural areas in the region where the demand of food, feed and biomass is located without outsourcing the land consumption and harming global ecosystem services as shown for the global soybean trade (Boerema et al., 2016). Furthermore, this study was conducted as case study to provide knowledge for the restoration of other marginal and neglected sites to examine the possibility for its preparation for agricultural crop production. The project as a whole consisted of seven sites with contrasting problems, such as drought, salt spray, heavy metal and organic pollution (M3). Therefore, the gentle restoration of large areas, that have been set aside in the past, will be a valuable tool contributing to mitigate the pressure on the most fertile soils which are endangered to be at least partly degraded through a non-sustainable intensive management.

4.2 Replacing Mineral Fertilizer by Organic Amendments

4.2.1 General Information for Amendment Application

The transformation of conventional to more sustainable agriculture will require improved sustainable cropping systems to restore, maintain or even enhance soil health and ensure plant performance on already existing arable fields, but it will also need to restore previously set aside agricultural areas. Besides the incorporation of plant residues which were grown on the arable field itself, the well balanced application of fertilizer is essential to provide sufficient NO_3^- and other macro- and micronutrients to maintain agricultural production without depleting the soil. Organic amendments obtained from farm residues within the concept of circular bioeconomy have multiple

advantages in this respect, even for the restoration of marginal soils (M3). Future sustainable agricultural management must focus on finding appropriate amendment combinations and to optimize their compositions and application rates to provide sufficient nutrients for improved plant performance while protecting natural soil functions.

In this context, it is important to mention that organic nutrients must first decompose to cycle through their inorganic form to unfold their nutritional potential and thus to become available for plants (Dibb, 2002). This mineralization process usually takes some time and depends on many different parameters like type and characteristics of the organic amendment, microbial activity, soil type, soil moisture and soil temperature (Dey et al., 2019). Considering the delay between application and plant uptake and the high nutrient variability within organic amendments (Timsina, 2018), it may even be recommended to combine organic with inorganic fertilizers to find optimal compositions for specific soils and specific cultivars. It is important to optimize fertilization strategies to decrease nutrient losses and improve the nutrient efficiency with the selection of the right source, right rate, right time and right place of amendment application on arable fields (M3) (Ju and Zhang, 2017). The combination of organic and mineral fertilizer might moreover support smooth transformation of conventional, intense agriculture into a more sustainably sound farm management since it is likely to be more convincing to farmers, who have used mineral fertilizers since decades, if a slight transition would not play off one amendment against each other. Consequently, the requirement of mineral fertilizer still could be reduced and the related disadvantages after its application on arable fields (e.g. soil acidification, decrease of soil quality, contamination of groundwater etc.) and during its production (e.g. energy consumption, greenhouse gas emissions etc.) could be alleviated.

To provide a data set for such an approach, we analyzed various combinations of organic amendments with and without mineral fertilizer and their effects on plant growth and soil health. For this, we grew *Hordeum vulgare* L. in soils amended with selected spent mushroom substrates blended with digestates and straw, and various rates of biochar and mineral fertilizer. The aim was not only to mitigate the increasing environmental burdens directly related to the excessive application of mineral fertilizer on arable fields but also to contribute to the increasing amounts of residues within industrial food and energy production and to exemplify options for re-use and closed nutrient cycles on a farm to thus lower the ecological footprint of sustainable farming.

4.2.2 Influence on Plant Performance and Soil Nutrient Stocks

In a greenhouse study (M2) *H. vulgare* plants grew better across all different fertilizer treatments with the best performance when spent mushroom substrate pellets alone (P) or in combination with mineral fertilizer (P_MF50) were applied. This is highly interesting since it indicates that nutrients provided by the pellets alone were already sufficient to improve plant performance regardless of mineral fertilizer application. This highlights the advantage of combining appropriate amendments already during composting and optimizing their composition before their application on the field. The high nutrient and organic matter content of spent mushroom substrate (Paula et al., 2019), the highly plant available nutrients in the digestate (Tambone et al., 2010) in combination with the capability of straw to buffer pH and temporal excess nitrogen (Reichel et al., 2018; Hao et al., 2020) seem to be highly favorable for plant growth. Hence, this combination seems promising to minimize or even replace mineral fertilizer for barley cultivation, at least under the conditions given. Of course, enhanced crop yields have repeatedly been reported when combining organic amendments with mineral fertilizer (Ehmann et al., 2018; Timsina, 2018; Zhao et al., 2016; Zhong et al., 2010), but the individual evaluation of fertilizer combinations for different soils and even cultivars is decisive to optimize for site-specific nutrient management.

In this context, we observed slightly decreasing plant biomass with increasing biochar concentrations, emphasizing the importance of leveling amendment application to correct rates to optimize beneficial effects on plant performance (Liu et al., 2018b). Increased NO_3^- concentrations remaining in soils amended with higher amounts of biochar are partially induced by higher rates of applied mineral fertilizer and furthermore by the retention capacity of biochar. The latter has been already described for biochar amended soils (Haider et al., 2017; Prendergast-Miller et al., 2014). Together with the improved water holding capacity and the prevention of nutrient leaching (Haider et al., 2017; Ulyett et al., 2014) this might be of interest especially for arid and nutrient depleted soils and could furthermore help plants to adapt to more frequent climate extremes (e.g. droughts, heavy rainfalls) in future sustainable cropping systems. However, Haider et al. (2017) did not find alleviating effects on nitrogen uptake limitation under drought scenarios and no improvement of crop yields at least for non-nutrient-loaded biochar application. Nevertheless, the high alkalinity of biochar, in the present case pH of 8.5 ± 0.1, can neutralize or even reverse soil acidification which highlights another important advantage when applying biochar with mineral

fertilizers or incorporating it into acidic soils (Dai et al., 2017; Van Zwieten, 2018). Anderson et al. (2011) furthermore reported the potential to reduce N_2O emissions, promote phosphate solubilizing bacteria, and even to decrease bacterial plant pathogens when biochar is applied. It might be concluded that biochar can be a promising soil amendment which might contribute to more sustainable crop production at low cost, especially when it is obtained from energy production of plant and animal wastes and when site-specific application strategies are well planned (M3). Still, its combination with other organic amendments and bacterial inoculants is recommended to add beneficial effects for improving soil quality and plant performance but should be further studied in long-term field experiments.

4.2.3 Microbial Responses of Organic Amendment Application

Besides the soil nitrogen pool also the C/N ratio and several other important soil quality indicators need to be considered to evaluate effects after fertilizer application. In our study, the different C/N ratios of the selected fertilizer combinations did not, contrary to our expectations, lead to an alteration of microbial biomass nitrogen (MBN) or microbial biomass C/N ratio in the soil-rhizosphere mixture. Consequently, no changes of microbial N immobilization were induced that would influence the nitrogen-transforming flows (Murphy et al., 2000). Similarly, further indicators of soil quality, like the overall microbial activity, the metabolic diversity of the microbial community as well as the activity of three extracellular enzymes (EEA's: β-glucosidase, acid phosphatase and β-N-acetylhexosaminidase) were not affected by the selected fertilizer combinations. Contrarily, many studies reported increasing enzyme activities, that might indicate improved soil health and accelerated nutrient transformation (Caldwell, 2005), after the input of organic matter introduced by organic amendments (e.g. biochar, straw, manure) (Li et al., 2018; Zhao et al., 2016; Zhong et al., 2010; Zhou et al., 2019). However, these results should be interpreted carefully, since only some potential enzyme activities were measured while soil functions are the result of a great variety of different enzymatic reactions (Nannipieri et al., 2012). Nevertheless, the stable microbial and enzymatic activities are indicators for maintained soil health and indicate together with the changes observed within the bacterial composition (e.g. on family level) functional redundancy within the soil-rhizosphere mixture (Louca et al., 2018). Pan et al. (2014) reported similar effects of stabilized microbial functions despite changes in the microbial composition after long-term fertilizer application also in grassland

soil. This important finding reveals that changes of the bacterial composition do not necessarily impact important microbial functions negatively and, similar to effects observed during the grassland conversion (M1), the resilience of the given soil to maintain its quality and function when appropriate management practices are applied.

However, the strong shift of the bacterial composition (e.g. β-diversity, species evenness and comparative abundance analysis) between initial and final sampling as well as changes of the extracellular enzyme activities observed during the greenhouse experiment (M2) indicate a strong influence of plant growth and associated mechanisms (e.g. root exudation) on the soil-rhizosphere continuum. The influence of root exudates on shaping microbial activity and composition in soil and rhizosphere (Nannipieri et al., 2008; Wang et al., 2017) as well as changes within rhizodeposition induced by plant species, growth and root development have already been shown (Brimecombe et al., 2007; Philippot et al., 2013). This emphasizes the importance of taking the role of a distinct plant species, its development stage, and specific belowground parameters (e.g. root exudates) into account when analyzing different fertilizer combinations and aiming to predict their effects on soil-rhizosphere microbiota. More detailed analysis of root exudates will furthermore support the implementation of bacterial inoculants since root exudates are known to influence their efficiency (Souza et al., 2015) and thus plant performance. This shows once more that a holistically and site-specific in-depth analysis of organic amendments and biological inoculants will be required to unravel plant-microbe interactions and agricultural productivity. It further highlights the limitations of the traditional focus on physical-chemical parameters to assess soil health and quality (Gómez-Sagasti et al., 2018), and emphasizes the importance of evaluating biological soil criteria. The microbial composition in soil is essential for agricultural management practices since it influences the microbial response after e.g. amendment application or grassland conversion (Fernandez et al., 2016) and aids plants (e.g. barley) to cope with abiotic stress (Yang et al., 2020).

Although it has to be mentioned that fungi enter another essential symbiosis between microbes and plants, their role could not be analyzed within the project (M1 and M2).

The most dominant bacterial phyla within the greenhouse study (M2) were very similar to results of the grassland conversion (M1) and are known to be common in the soil environment (Fierer et al., 2017; Lauber et al., 2009). Neither grassland conversion (M1) nor different

fertilizer combinations (M2) had a strong influence on the bacterial composition on phylum level. In contrast, Buée et al. (2009) found strong variations of the most abundant phyla, in particular of *Proteobacteria*, *Actinobacteria* and *Acidobacteria*, between studies and treatments. However, a significant change within the fertilizer study (M2) was found when comparing the initial soil with the final soil-rhizosphere mixture and was mainly assigned to *H. vulgare* growth, root development and associated mechanisms (e.g. root exudation). In more detail, we observed a strong increase of *Acidobacteria* and a strong decrease of *Actinobacteria*, *Chloroflexi* and *Bacteroidetes* in the final soil-rhizosphere mixture compared to the initial bulk soil. Decreasing abundances of *Chloroflexi* can be explained by findings of Bulgarelli et al. (2015) who found *Chloroflexi* virtually excluded from rhizosphere and roots of different barley varieties. In contrast to this, the strongly increasing abundances of *Acidobacteria* in our final soil-rhizosphere mixture might also indicate effects of barley growth and root development since Buée et al. (2009) found *Acidobacteria* to dominate the rhizosphere of various plant species. This strong acidobacterial increase might furthermore be supported by the input of organic and inorganic nutrients since its abundance has been positively correlated to organic matter and carbon availability (Kielak et al., 2016; Navarrete et al., 2013). Moreover, the high root density in the pots is likely to promote high carbon content through rhizodeposition (Philippot et al., 2013) which indicates once more the strong influence of plant development on shaping the bacterial composition in a soil-rhizosphere mixture.

Similarly, to the dynamics of the bacterial composition on phyla level, the observation of bacterial families revealed the most pronounced differences when comparing the initial soil with the final soil-rhizosphere mixture. Among the most pronounced differences was an increase for the families *Xanthobacteraceae*, *Mycobacteriaceae*, and *Pyrinomonadaceae* and a decrease *Nitrosomonadaceae*, *Chitinophagaceae*, *Xanthomonadaceae*, and *Burkholderiaceae* towards the final sampling. Decreasing abundances of the ammonia-oxidizing family *Nitrosomonadaceae* during the cultivation of cover crops and the application of organic fertilizer have already been reported by Fernandez et al. (2016) who also described, similar to our findings, the stronger effects of the plant's rhizosphere on shaping the bacterial composition than the application of organic amendments. It can hence be concluded that also the bacterial composition on family level is, similar to our finding on phylum level, strongly

influenced by plant development and its mechanisms. In contrast, Semenov et al. (2020) found long-term fertilization more important for shaping prokaryotic communities in soil and rhizosphere within agricultural ecosystems than specific crop species (maize, potato, white mustard).

However, although effects of plant developmental stage and associated mechanisms (e.g. root exudation) were dominating the changes of the bacterial composition in our experiment, also minor changes were observed between the different fertilizer treatments. These were most pronounced after treatment with the highest amount of mineral fertilizer in combination with biochar (B_MF140). This treatment caused the lowest species evenness as well as the lowest pH which might be induced by the highest rate of mineral fertilizer and could indicate one of its disadvantages, namely soil acidification. The highest amount of NO_3^- for this treatment remaining in the end of the experiment (M2) might not only be explained by the highest input of mineral fertilizer but also by the retention capacity of biochar indicating one of its advantages as already described above. Compared to other treatments the highest relative abundance of *Xanthobacteraceae* and *Mycobacteriaceae* and the lowest for *Haliangiaceae* was observed of which the latter two families have been found to be associated to NO_3^- (M2). Similarly, Anderson et al. (2011) found *Mycobacteriaceae* (specifically *Mycobacterium*) enriched in biochar amended soils and highlighted their role as NO_3^- reducers helping to balance the nutrient pool in soil. Since biochar carbon is largely unavailable for plants these authors found mainly altered soil physiochemical properties responsible for shifts in the soil microbial composition.

Altogether, this indicates that besides plant development also differences in the fertilizer composition and application rate can influence the bacterial composition in soil especially if higher rates of mineral fertilizer together with higher rates of biochar are applied. This highlights that besides finding the best combination of different organic amendments and inorganic fertilizers and to optimize its composition also the right application rate is essential for not only improving plant performance but also maintaining soil quality and health in particular when aiming in the restoration of marginal soils. Nevertheless, we found the influence of plant and root development and also the indigenous soil microbiota most important for the provision of soil quality and its functions.

5 Conclusion and Recommendations

Facing the increasing pressure on arable soils and on agricultural productivity caused by the demands of an ever growing world population and a rapidly changing climate, alternatives for the excessive application of mineral fertilizer and other detrimental conventional management practices are needed to ensure global food security while protecting important ecosystem services. Integrated, organic and sustainable agricultural management strategies have to be implemented to not only minimize negative impacts of conventional treatment on agricultural sites but even enhance quality and resilience of natural ecosystems (M3). In this context, we examined the application of various soil amendments in a greenhouse experiment, aiming to improve performance of *H. vulgare* plants and to maintain or even enhance soil quality and functioning (M2). Moreover, we studied the gentle restoration of marginal grassland which had previously been set-aside to alleviate the pressure on existing fields and counteract the increasing decline of fertile soils (M1). The aim was to evaluate such agricultural management practices holistically, emphasizing especially on the analysis of plant-microbe interactions and thus to provide knowledge for future measures at similar sites.

Overall, this thesis demonstrates the great potential of site adapted management practices, such as the gentle grassland conversion (M1) and addition of suitable organic amendments (M2), to restore marginal land while also protecting soil health and important soil functions (M3). Strong short-term effects of soil nutrient stocks and on soil bacteria during the grassland conversion to arable land (M1) and after application of selected fertilizer combinations in the *H. vulgare* greenhouse experiment (M2) could be observed. Interestingly, although a clear influence of agricultural management practices (M1) and plant development stage (M2) on shaping the highly diverse bacterial community structure was found, the bacterial phyla remained almost unaffected during the conversion (M1) and even from the influence of different fertilizers (M2) which is a first indication of gentle management strategies and the resilience of the given soil. Nevertheless, strong bacterial changes have been observed on family and species level (M1 and M2) which were mainly affected by decomposition processes (M1), plant development stage (M2), enriched NO_3^- (M1 and M2), decreasing DOC/TNb ratio (M1), and the application of higher amounts of mineral fertilizer in combination with biochar (M2). Although pH is considered to be one of the major drivers of soil microbiota, neither the grassland conversion (M1) nor fertilizer application (M2) affected

pH in our studies significantly. Thus we found the abovementioned parameters, in particular decomposition processes (M1) and plant development (M2), more important for shaping bacterial composition when site-specific management is well planned. The slight decrease of Simpson's index of diversity (M1) and Pielou's species evenness (M2) towards the final samplings indicates a shift to more dominant species which was fortunately found to be dominated by higher abundance of beneficial bacteria, such as *Massilia* which can stimulate plant growth (M1), and *Lysobacter* that support the biological control of plant diseases (M1). The higher occurrence of NO_3^- reducers (*Massilia* (M1), *Mycobacteriaceae* (M2)) in line with the highest NO_3^- concentrations in soil shows the adaptation of soil bacteria on changing conditions which helps to balance the soil nutrient pool. Noteworthy, the unaffected microbial activity, potential enzyme activity and metabolic diversity of the microbial community among the different fertilizer treatments (M2) in conjunction with the bacterial changes observed, e.g. on family level, indicate functional redundancy. This important finding proves that soil functions are maintained despite changes within soil bacteria in the greenhouse experiment (M2). Both, the higher abundance of beneficial microbes (M1 and M2) and the functional redundancy (M2) are good indicators for the resilience and quality of the given soil and highlight the great potential for appropriate fertilizer combinations (M2) and even for the gentle transformation of marginal grassland (M1) to contribute to integrated and organic agricultural management.

It is important to mention, that during the conversion experiment the mineralization after breaking-up the grassland in combination with the biological nitrogen fixation of *V. faba* and its subsequent incorporation mobilized the NO_3^- pool in soil sufficiently even for future cropping of arable plants (M1). This confirms the possibility of appropriate agricultural management, such as leguminous intercropping and green manure application, to introduce huge amounts of plant available nitrogen into soil without applying additional synthetic nitrogen fertilizer from industrial processes, which might endanger soil health (e.g. soil acidification). Together with the enrichment of beneficial bacteria (e.g. *Massilia, Lysobacter, Rhizobiales*) this demonstrates the great success of the gentle restoration strategy of set-aside grassland for future crop cultivation. The conversion experiment thus provides important knowledge how to restore and unlock the potential also for other marginal sites and consequently contributes to the expansion of arable fields, thus lowering the pressure on the most fertile soils.

In the greenhouse experiment, *H. vulgare* plants grew well under all fertilizer combinations, without revealing significant differences between organic amendments alone or in combination with mineral fertilizer (M2). This confirms the possibility of using appropriate organic amendments, in particular pelletized spent mushroom substrate blended with digestate and straw, to minimize or even replace inputs of mineral fertilizer in environmentally sound agriculture. The very good performance of these pellets demonstrates how former waste products from food (spent mushroom substrate) and energy production (digestate) can be transformed into renewable resources (Phan and Sabaratnam, 2012). Similarly, the application of biochar from energy production enables improved agricultural production and thus helps to mitigate its impact on global warming if its production and application is thoroughly planned (Qambrani et al., 2017). Interestingly, neither the strongly varying C/N ratios of the amendments influenced the microbial C/N ratio and thus nitrogen flows nor did the application with and without mineral fertilizer change microbial activities. This indicates once more a resilient soil and moreover the importance of taking the indigenous soil microbiota into account when assessing agricultural soil for its suitability for sustainable crop production. Even the potential extracellular enzyme activity remained unaffected by the application of various amendments but not from plant growth, root development and associated mechanisms. This emphasizes the importance of site-specific and individual management practices (e.g. organic amendment application) for different plant species and agricultural soils to foster distinct effects on the soil-plant-microbe interaction and thus on agricultural productivity. Under the conditions of our study only beneficial effects of organic amendment application were observed. The findings furthermore highlight the significance of the plant species, its development stage and belowground parameters (e.g. root exudates) when analyzing fertilization effects on microbial composition and activity in soil.

As shown above, well selected combinations, compositions and application rates of organic amendments are promising for improved plant performance without harming natural soil functions. It is thus recommendable to combine different features of various amendments to optimize their physical, chemical and biological properties and thus their nutrition potential for different cultivars and specific soils already during production (e.g. composting) or prior to their application on arable or depleted soils. However, although such optimized organic amendments will play an important role in the transformation to sustainable farming systems not

a single approach will ensure sufficient food, feed and biomass production alone (Reganold and Wachter, 2016). It is thus essential to combine organic farming with innovative (e.g. precision agriculture) and integrated farming techniques to ensure agricultural production while reducing the amount of agrochemicals that are applied onto arable fields. The aim is not only to replace mineral fertilizer and other agrochemical compounds on arable fields but to use them wisely, and contribute to the increasing amounts of organic waste derived from food and energy production preferably from the same farm or its direct vicinity to save transportation costs and reduce the ecological footprint.

However, we recommend long-term field studies to confirm our findings that suitable fertilizer combinations and gentle management strategies can contribute to a more sustainable agricultural crop production including the protection of soils and the restoration of marginal land. We moreover suggest the field application of selected members of the genus *Massilia, Lysobacter* and the order *Rhiozobiales* as bacterial inoculants to promote soil health and plant performance and thus to support agricultural productivity biologically while reducing inputs of agrochemicals on arable fields. Making use of such bacterial inoculants in combination with appropriate organic amendments and microbiome-based farming practices seems promising to improve plant-microbe interactions with beneficial effects on soil health and agricultural production (Compant et al., 2019). We moreover suggest that these bacteria might be good biological indicators for soil quality and can reflect anthropogenic influences as shown by Hermans et al. (2017) for *Gaiellaceae* and other soil bacteria. In addition, also the knowledge of local farmers about plants as indicators for soil quality should be combined and supplemented with remote sensing, geographic information systems (GIS) and molecular data of the soil-plant-microbe interaction to explain mechanisms of soil biota even on landscape scale (Barrios, 2007). The present thesis already demonstrates the great potential of molecular analysis and its relevance in a holistic evaluation of agricultural management for improving soil quality and functioning and plant performance.

We furthermore recommend the development of a framework to support farmers, decision makers and all stakeholders which are involved in agricultural management, to optimize fertilizing strategies with lower impact on the environment (M3). Therefore, future developments must imply a global or at least European standardization of analytical procedures for the evaluation of soil amendments (e.g. biochar, digestate) according their potential to enhance crop productivity and

foster important ecosystem services (e.g. nutrient cycling, carbon sequestration, water retention) contributing moreover to mitigate the agricultural influence on climate change (M3). Still, region-specific transformation processes should be supported which are taking the local availability of organic residues and additives into account for optimizing the amendments sustainability (Barthod et al., 2018, Matovic, 2011). Nevertheless, site-specific and interdisciplinary evaluation of agricultural management will gain importance to reveal in-depth knowledge about the benefits after applying optimized fertilizer combinations or converting marginal land and to accompany the transformation to a more sustainably sound agriculture adapted to climate change. The aim is not longer to simply maximize crop production, but to optimize management strategies also with regard to environmental and social implications, and to prevent the further degradation of our production basis (M3).

6 Outlook and Perspectives

In the not too distant future the combination of multiple advantages of further developed traditional farming practices and novel agricultural technologies will lower the agricultural impact on the environment while also maintaining or even enhancing agricultural productivity. Especially, the combination of precision farming tools with aspects of integrated and organic agriculture seem to be promising to improve site-specific management decisions of farmers (Schmidhalter et al., 2008). In addition, it will be essential to evaluate in a holistic approach whether e.g. the application of organic amendments or conversion of marginal land is both, ecologically as well as economically sound at the time and region of application and further on global scale. A repository of data will be needed which can be obtained in particular via the analyses of soil and plant processes through e.g. molecular barcoding and the additional application of proximal and remote sensors. Assuming a smart data collection, these datasets can and should be combined to obtain interdisciplinary knowledge for the site specific evaluation to develop novel approaches for sustainable management to e.g. unlock the potential of marginal or neglected land (see Figure 2) or apply appropriate organic amendments.

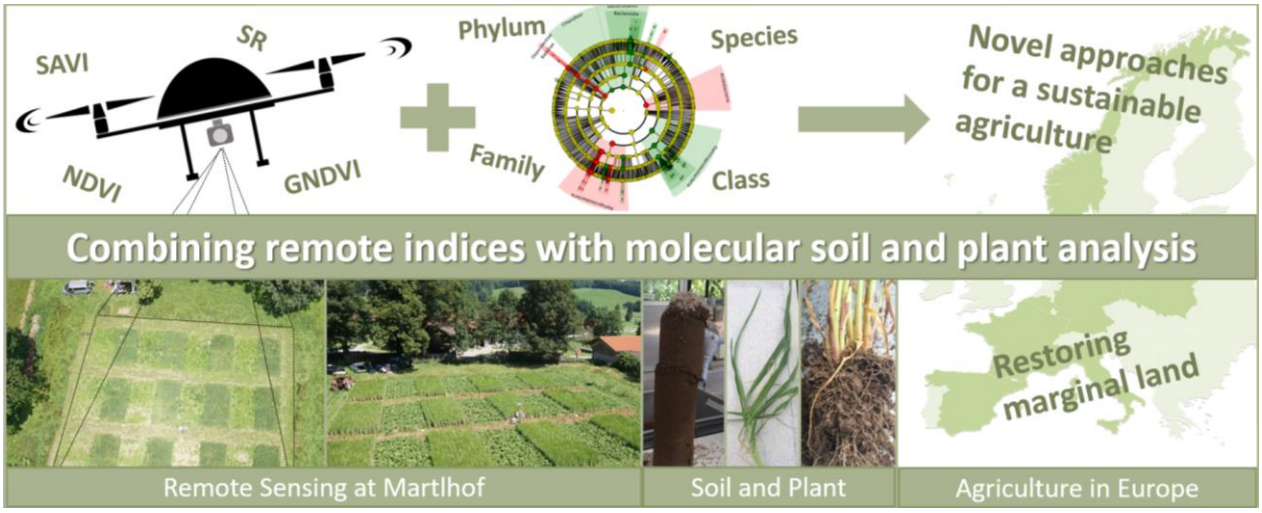

Figure 2 Illustration of a future scenario: Combination of remote indices with data obtained from molecular barcoding of soil and plant processes to provide knowledge about plant-microbe interaction and develop novel approaches for a sustainable agriculture in Europe.

Given the complexity of such multi-parametric evaluation of soil health, plant performance and microbial composition, intelligent models need to be implemented that enable the handling of these large datasets and generate beneficial guidance for farmers, decision makers and all stakeholders that are involved in shaping more sustainable agriculture. To filter essential mechanisms of the soil-plant-microbe interaction that can be influenced by appropriate farm management such novel models will need to include fuzzy logic approaches (M3) and might in future even be supported by artificial intelligence. To date, FACCE-JPI already provides the knowledge hub MACSUR (Modelling European Agriculture with Climate Change for Food Security, https://www.macsur.eu/) which aims to align national research and harmonize various modelling systems to improve the methodology for integrative interdisciplinary modelling of European agriculture (Gøtke et al., 2016) and thus to support sustainable agricultural management.

Such sustainable management must enhance agricultural production with focus on optimized fertilization strategies, diminished soil nutrient losses and greenhouse gas emissions as well as reduced water consumption to feed the growing world population and mitigate the impact on global environment and climate change (Hunter et al., 2017). Application of organic fertilizers obtained from circular processes, using plant growth-promoting bacterial inoculants as well as integrated pest control are indispensable tools to be developed and to be combined with improved recommendations for crop rotation, harvest and postharvest strategies (Schröder et al., 2019). Reclamation of waste water could moreover mitigate water shortage in particular in arid areas if its composition is of sufficient quality (Schröder et al., 2019). Furthermore, plant breeding supported by high-throughput phenotyping should aim at improving plant-microbe interactions (Compant et al., 2019) and must encompass unfavorable environmental conditions (e.g. drought, heavy rainfalls) caused by climate change. In addition, well-planned agroforestry systems and particular evergreen agriculture are promising for improving soil fertility and crop yields especially under tropical and sub-tropical climate (Timsina, 2018).

If such sustainable farming practices will be supported by suitable governmental incentives, farmers might be able to conserve natural ecosystems providing essential ecosystem services and not only ensure agricultural production but even provide social and cultural benefits (M3). Therefore, the European Union should transform its Common Agricultural Policy (CAP), which is mainly subsidizing agricultural area and thus the enlargement of farms, to support integrated

organic agricultural production embedded in a circular bioeconomy in which also small-scale farms can persist and implement site-specific sustainable management at high agricultural productivity and protection of central ecosystem services. European agricultural policy must succeed to promote tools, methods and solutions that support such sustainable agricultural systems to implement the global agendas for the Sustainable Development Goals of the United Nations (Gøtke et al., 2016) and thus to improve our common future.

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V. Appendix

i. Appendix M1 (Manuscript I)

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Under temperate climate, the conversion of grassland to arable land affects soil nutrient stocks and bacteria in a short term

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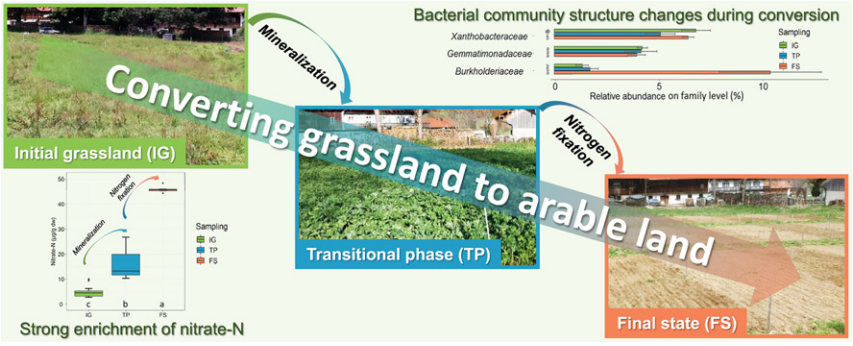
HIGHLIGHTS

- Strong increase of nitrate-N due to mineralization and biological nitrogen fixation
- Bacterial composition on phylum level and bacterial richness remain quite stable.
- Beta-diversity analysis indicates changes due to the management practices.
- High abundance of *Massilia* and *Lysobacter* after incorporation and decay of *V. faba*
- Gentle management strategies can replace the input of mineral fertilizer.

GRAPHICAL ABSTRACT



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ABSTRACT

Projected population growth and climate change will make it inevitable to convert neglected and marginal land into productive arable land. We investigate the influence of agricultural management practices on nutrient stocks and soil functions during the conversion of former extensively used grassland to arable land. Effects of grassland removal, tillage, intercropping with faba bean (*Vicia faba*) and its later incorporation were studied with respect to soil properties and bacterial community structure. Therefore, composite samples were collected with a core sampler from the topsoil (0–20 cm) in (a) the initial grassland, (b) the transitional phase during the vegetation period of *V. faba*, (c) after ploughing the legume in, and (d) untreated controls. In all samples, nitrate-N, ammonium-N, dissolved organic carbon (DOC) and total nitrogen bound (TNb) were analyzed and comparisons of the bacterial community structure after 16S-amplicon sequencing were performed to assess soil functions. Mineralization after grassland conversion followed by the biological nitrogen fixation of broad beans enhanced the nitrate-N content in bulk soil from 4 to almost 50 μg N g^{-1} *dw*. Bacterial community structure on phylum level in bulk soil was dominated by *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes* and remained almost stable. However, alpha and beta-diversity analysis revealed a change of the bacterial composition at the final state of the conversion. This change was primarily driven by increasing abundances of the genera *Massilia* and *Lysobacter*, both members of the *Proteobacteria*, after the decay of the leguminous plant residues.

Abbreviations: ASV, amplicon sequence variant; *bp*, base pairs; *BSA*, Bovine serum albumin; *C*, carbon; *CG*, control grassland; *Chl*, chlorophyll; *DNA*, deoxyribonucleic acid; *DOC*, dissolved organic carbon; *dw*, dry weight; *FS*, final state; *fw*, fresh weight; *FACCE-JPI*, Joint Programming Initiative on Agriculture, Food Security and Climate Change; *IG*, initial grassland; *INTENSE*, Intensify production, transform biomass to energy and novel goods and protect soils in Europe; *N*, nitrogen; *NA*, not assigned; *NMDS*, non-metric multidimensional scaling; *spp*, species pluralis; *PCR*, polymerase chain reaction; *rRNA*, ribosomal ribonucleic acid; *TNb*, total nitrogen bound; *TP*, transitional phase.

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Furthermore, increasing abundances of the family *Gaiellaceae* and its genus *Gaiella* fostered this change and were related to the decreasing carbon to nitrogen ratio. In short, gentle management strategies could replace the input of mineral fertilizer with the aim to contribute to future sustainable and intensified production even on converted grassland.

1. Introduction

Projections show that feeding a world population of 9.1 billion people will require 70% increase in global food production by 2050 (FAO, 2009). In particular, increased plant production will be necessary to ensure food and feed supply, and to respond to the need for biomass as renewable energy and industrial feedstock application. In order to meet these challenges, we must improve biomass production and utilization to satisfy the social, economic, and environmental demand of the growing population (Schröder et al., 2018). Including neglected and upgrading marginal sites will be unavoidable and the gentle management of such sites indispensable to maintain or even improve soil quality, functionality, and health and thus to contribute to a more sustainable agriculture (Lal, 2016; Schröder et al., 2019).

In contrast, mineral fertilizers have been used since decades to increase plant production in conventional farming with diverse effects on fertility and physical properties of soils (Aggelides and Londra, 2000; Ahmed et al., 2017). To mitigate resulting negative effects, techniques have to be developed or rediscovered to replace or at least minimize the input of inorganic fertilizer to commonly used agricultural farmlands and also to lower the release of nitrate-N into groundwater (EC, 2000). Therefore, undersowing of leguminous species (Schröder et al., 2008), intercropping of faba bean (Fan et al., 2006), incorporation of leguminous plant residues (Ordóñez-Fernández et al., 2018) as well as organic amendments have been proven to be beneficial and also to enhance crop yield (Diacono and Montemurro, 2011; Scotti et al., 2015; Lori et al., 2018).

In addition, it will be inevitable to convert neglected and marginal land into productive arable land. Even the conversion of poor grassland into economically attractive cropland may be considered if it can be reached in a sustainable manner. To do this, nutrient pools need to be stabilized and the prevailing bacterial community structures and activities have to be maintained or even enhanced. It will be essential to face possible negative effects following grassland break-up like increased nitrogen losses, due to nitrate leaching and nitrous oxide emissions, following the mineralization of soil organic nitrogen and the decomposition of grass residues (Buchen et al., 2017). To compensate these effects, intercropping of faba bean seems to be promising since it not only facilitates atmospheric nitrogen fixation and thus improves soil fertility (Stagnari et al., 2017) but also reduces nitrate leaching if it is used as cover crop (Plaza-Bonilla et al., 2015). However, little is known about the influence of intercropping legumes and their later incorporation during grassland conversion on bacterial community structure.

Several studies revealed the importance of bacterial community structure on ecosystem services such as nutrient cycling in soil (Barrios, 2007; Zhong et al., 2010; Kaiser et al., 2016). It is crucial to know that climate (Sheik et al., 2011) and different land use intensities (Estendorfer et al., 2017) can change bacterial communities. Since the intervention in ecosystems (e.g. the conversion of grassland to arable land) can also disturb and change bacterial diversity and composition (Gatica and Cytryn, 2013; Carbonetto et al., 2014; Hartmann et al., 2015) such conversion must be well planned and monitored.

Being part of an interdisciplinary project, we hypothesize that neglected land can be re-activated as high-value cropland without losses in nutrient pools or decreases in ecosystem services. To test this, the grassland of a small-scale dairy farm in southern Bavaria was converted into cropland and broad bean was used as intercrop and later incorporated into the soil. We aimed to assess the early consequences of such land use change on nutrient availability and bacterial community structure and thus to contribute to a more sustainable and intensified agriculture. The focus was hereby on studying the changes induced by mineralization processes during grassland conversion, the nitrogen fixation of *V. faba*, and degradation processes after incorporating its residues into the soil. Finally, it was intended to provide enough nitrate-N for further cropping of arable plants and thus to replace any additional input of mineral fertilizer.

2. Materials and methods

2.1. Site description

The study site is based at Martlhof, a traditional small-scale dairy farm, raising sheep and pigs on pasture, on former extensively used grassland, in Ostin am Tegernsee (Bavaria, Germany, 47° 44′ 37.30″ N and 11° 45′ 38.32″ E). The field trial (1 ha) is located 784 m above sea level with a gently sloping relief. Climatic conditions are in the transition zone of the warm-temperate climate of Western Europe and the colder continental climate of Eastern Europe. A mean annual precipitation of 991 mm, a mean annual temperature of 7.5 °C, and a mean annual sunshine duration of 1571 h characterize the climate in this region. The site's bedrock is calcareous, the colluvial topsoil contains 28.2% sand, 43.1% silt, and 28.8% clay. Its texture has been classified as clayey loam with an average pH ranging from 5.2–5.6.

2.2. Experimental layout and agricultural management practices

To analyze the effects of grassland transformation on bacterial community structure, a short-term field trial was started in May 2016. Therefore, the experimental field (32 × 32 m) as a whole was subdivided into six subplots of 10.7 × 14 m (see Supplementary Fig. S1). The six subplots (I, II, III, IV, V, and VI) were separated from the beginning of the experiment and complemented with four untreated grassland controls of 8 × 4 m size each. Randomized sampling was performed in the center of each plot to avoid transition effects between the subplots (composite of 12 subsamples). A phytosociological survey of the grassland was performed according to Ellenberg (1992). The Ellenberg indicator values (e.g. individuality, sociability, temperature, nitrogen) are given in Supplementary Table S1. The grass scar was mechanically mulched and the residual green cover was incorporated into the soil. Following the milling of the top soil (12 cm) broad beans (*Vicia faba* L.) were sown (200 seeds/m^2) as cover crop. This was done to homogenize the field area, to facilitate biological fixation of nitrogen, and at the same time to avoid weed invasion and leaching of nutrients. In April 2017, the leguminous plant residues were incorporated and in May the top soil was tilled to a depth of 18 cm using a three-furrow turning plough. After milling with a harrow, the field reached its final state of transition.

2.3. Soil and plant sampling procedure

Soil sampling to analyze soil properties and bacterial community structure up to a depth of 20 cm was performed using a core sampler. Bacterial analysis was done for a total sample number of 20. Therefore, the 12 subsamples of each plot were pooled and homogenized. The samples were sieved (2 mm), frozen on dry ice and subsequently stored

at −80 °C for later bacterial analysis. The soil samples used for analysis of nitrate, ammonia, total nitrogen bound, dissolved organic carbon and pH were stored at 4 °C. Moisture and temperature were measured on the field using a time domain reflectometer UMP-1 BTim (Umwelt-Geräte-Technik GmbH, Müncheberg, Germany). Sampling was performed at four different sampling dates. Sampling 1 (IG) in July 2016 describes the initial status of the grassland (Supplementary Fig. S1). Sampling 2 (TP) was performed in November 2016 during the vegetative period of *V. faba* and describes the transitional phase. Sampling 3 (FS) was conducted in June 2017, describing the final state of the conversion to arable plots after incorporation of the leguminous plant residues. Additionally, Sampling 4 (CG) was accomplished in August 2017 and describes the status of the grassland without any management practices and acts as control, directly adjacent to the converted plots. Additionally, in TP, six plant samples of *V. faba* were taken from each plot to analyze the content of pigments (Chl *a*, Chl *b* and total carotenoids) as well as the plant fresh weight and height.

2.4. Nutrient stocks (DOC, TNb, nitrate-N and ammonium-N) and pH

Dissolved organic carbon (DOC) and total nitrogen bound (TNb) in bulk soil were extracted from 5 g of field fresh samples using 20 mL of 0.01 M $CaCl_2$. After shaking the samples for 45 min on a horizontal shaker the samples were filtered through a Whatman folded filter (type 595, diameter 110 mm, GE Healthcare, Buckinghamshire, United Kingdom). TNb and DOC were measured on a DIMATOC®2000 (DIMATEC, Langenhagen, Germany). Concentrations of nitrate (NO^{3-}-N) and ammonium (NH^{4+}-N) were analyzed photometrically by continuous flow measurements using an autoanalyzer (CFA-SAN Plus, Skalar Analytik, Erkelenz, Germany). To determine the gravimetric water content, subsamples of the bulk soil were dried for 24 h at 105 °C. Soil pH measurements followed the guidelines of the OECD (ISO 10390, 2005) adding 25 mL of 0.01 M $CaCl_2$ to 5 g of bulk soil samples.

2.5. Pigment analysis

Chlorophylls (Chl *a* and Chl *b*) and total carotenoids of *V. faba* plants were analyzed following the protocol of Lichtenthaler and Buschman (2001), slightly modified by Obermeier et al. (2015) (see legend of Supplementary Table S2).

2.6. Nucleic acid extraction

DNA was extracted from 0.5 g of bulk soil (−80 °C) using the Fast DNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, United States) according the manufacturer's instructions. Negative controls were included using empty extraction tubes. DNA concentrations were measured in duplicates using Quant-iTPico™ Green® ds DNA assay Kit (Thermo Fisher Scientific, Waltham, United States) following the manufacturer's protocol. Measurements were performed at 520 nm using a SpectraMax Gemini EM Fluorescence Plate Reader Spectrometer (Molecular Devices, Ismaning, Germany). Non-target controls were used to correct for background fluorescence. All DNA extracts were stored at −80 °C for further usage.

2.7. 16S library preparation and Illumina Sequencing

Polymerase chain reaction (PCR) of the 16S rRNA region was performed on 1 ng of DNA extracts in triplicates using primer S-D-Bact-0008-a-S-16 (5′-AGAGTTTGATCMTGGC-3′) and primer S-D-Bact-0343-a-A-15 (5′-CTGCTGCCTYCCGTA-3′) to amplify the V1-V2 region (Klindworth et al., 2013). PCR conditions were the following: denaturation at 98 °C for 30 s, followed by 28 cycles each at 98 °C for 10 s (denaturation), 60 °C for 30 s (annealing) and 72 °C for 30 s (elongation), followed by 72 °C for 5 min (final elongation). A non-target control (NTC) and a positive control with the target gene were also performed following the same PCR conditions. The reaction mix contained 12.5 μL of NEBNext High-Fidelity Master Mix (New England Biolabs, Ipswich, United States), 5 pmol of each primer, 10.5 μL of DEPC water, 2.5 μL of 3% bovine serum albumin (BSA) and 1 ng of DNA extract. The quality of the PCR amplicons was checked on 1% agarose gels. Triplicate DNA reactions were pooled and purified using Agencourt®AMPure®XP kit (Beckman Coulter Inc., Webster, United States) according to the manufacturer's instructions (with the modification of using 78 μL beads for 60 μL of sample volume). DNA quantification and quality controls were performed using the DNF-473 standard sensitivity Kit (1 bp – 6000 bp) on a Fragment Analyzer device (Advanced Analytical Technologies GmbH, Heidelberg, Germany).

The Nextera XT Index Kit v2 (Illumina Inc., San Diego, United States) was used for indexing 10 ng of the 16S rRNA gene amplicons, according to the manufacturer's protocol. The PCR comprised initial denaturation with 98 °C for 30 s, followed by 8 cycles each at 98 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s, ending with a final elongation at 72 °C for 5 min. The indexed PCR products were purified, and quality as well as quantity were checked as described above. Next-generation sequencing was performed on 10 pM of indexed DNA, using the Illumina MiSeq platform (Illumina Inc., San Diego, United States).

2.8. Sequencing data analysis

To remove primers and adapters, the raw data from Illumina Sequencing was processed using the software AdapterRemoval (V. 2.1.7) (Lindgreen, 2012) separately for reverse and forward reads. For further processing, the R package DADA2 (V. 1.8.0) was used (Callahan et al., 2016). After checking read quality plots, quality filtering and trimming of forward reads was performed at 10 and 200 bp. For the reverse reads, trimming was done at 60 and 180 bp only. Remaining PhiX contaminations were removed during filtering. Subsequently, the samples were dereplicated and denoised before forward and reverse reads were merged. Thereafter, an ASV table was constructed and chimeras removed. Finally, taxonomic annotations of ASVs against the SILVA database version 128 (Quast et al., 2013) were performed.

Sequence data were imported to R (V. 3.5.1) (R Core Team, 2018) using the phyloseq package (V. 1.25.2) (McMurdie and Holmes, 2013), plotted using the ggplot2 package (V. 3.0.0) (Wickham, 2016) and statistically analyzed using the package agricolae (V. 1.2.8) (De Mendiburu, 2014). After filtering ASVs that were not assigned to bacteria (NA and eukaryota), chloroplasts and mitochondria, ASVs present in negative controls and ASVs that were present in only a single sample were removed. For the filtered data, a phylogenetic tree was calculated using the software RaxML-NG (V. 0.6.0) (Kozlov et al., 2018). Alpha diversity indices were plotted using the plot_richness function of the phyloseq package. Non-metric multidimensional scaling (NMDS) was performed on genus level to visualize dissimilarities between sampling points based on Bray-Curtis distances. NMDS was done for a reduction to two dimensions with a maximum of 500 tries using the vegan package (V. 2.4-6) (Oksanen et al., 2018). Ninety-five percent confidence ellipses were plotted for each sampling time. Tukey's post-hoc test based on Bray-Curtis dissimilarities in conjunction with a one-way ANOVA on relative abundances of phyla, orders, families, and genera was run to indicate which sampling time point differs significantly from others ($p < .05$). Relative abundances and standard deviation on phylum, order, family, and genus level are shown to indicate effects of the management practices as well as the homogeneity within the subplots of the experimental field. Further, a one-way ANOVA ($p < .05$) in conjunction with Tukey's post-hoc test was performed to analyze soil and plant data using basic R functions (R Core Team, 2018). The nucleotide sequence data are available in the NCBI Sequence Read Archive (SRA) (Leinonen et al., 2010) under the BioProject accession number PRJNA471669 (https://www.ncbi.nlm.nih.gov/sra/PRJNA471669).

3. Results

3.1. Climate, initial situation and soil properties

From January 2016 to October 2017, a typical temperature and precipitation pattern for the sub-continental climate prevailed at the experimental site (Supplementary Fig. S2). In the transitional phase (TP), four strong rain events (>30 mm/day) could be observed during the vegetation period of *V. faba*. Air temperatures at the sampling dates were 19.7 °C (IG – initial grassland), 5.0 °C (TP – transitional phase), 13.7 °C (FS – final state), and 26.3 °C (CG – control grassland).

Soil temperature and moisture were homogeneously distributed within the field plots at the different sampling dates (see Table 1). Furthermore, soil pH-values were homogeneous within the field plots and remained constant with only slight variations during the complete sampling period (5.4 ± 0.2). The basic inventory of the initial and the control situation of the grassland following the guidelines of Ellenberg values (Ellenberg, 1992) identified it as grassland of moderate quality, slightly moist without indications for salt stress (Supplementary Table S1).

3.2. Nutrient stocks (DOC, TNb, nitrate-N and ammonium-N)

A strong two-fold increase of DOC in bulk soil was observed from 15.3 ± 7.7 μg g^{-1} *dw* in IG to 30.9 ± 12.1 μg g^{-1} *dw* in TP (Table 2). Subsequently, DOC decreased to 12.5 ± 3.4 μg g^{-1} *dw* in FS. With 20.5 ± 5.8 μg g^{-1} *dw*, the DOC content in the control grassland (CG) was not significantly different from the initial situation (IG).

TNb increased three-fold from 5.2 ± 1.6 μg g^{-1} *dw* in IG to 15.2 ± 5.4 μg g^{-1} *dw* in TP and finally reached its maximum of 45.3 ± 5.0 μg g^{-1} *dw* at FS. In contrast, the contents of TNb in the control grassland (CG) showed no significant changes and remained with 8.1 ± 1.2 μg g^{-1} *dw* on the low level of the initial grassland (IG). A decrease of the DOC/TNb ratio from 3.0 in IG to 2.0 in TP and 0.3 in FS was observed during the transformation. The control grassland (CG) had DOC/TNb ratios similar to the initial grassland situation (IG).

Contents of nitrate-N exhibited similar trends compared to TNb (Fig. 1a). With 16.9 ± 5.9 μg nitrate-N g^{-1} *dw* a strong increase was already observed at TP to values three times higher than in IG (4.2 ± 1.1 μg nitrate-N g^{-1} *dw*). Subsequently, another three-fold increase (compared to TP) to 49.6 ± 5.5 μg nitrate-N g^{-1} *dw* in FS followed. Again, CG and IG had similar nitrate content (6.1 ± 1.9 μg nitrate-N g^{-1} *dw*).

Similar to TNb and nitrate-N, an increasing trend for ammonium-N was observed throughout the experiment (Fig. 1b). However, this increase was not significant and much less pronounced than the strong increase of nitrate-N and TNb. The ammonium-N content in the initial bulk soil doubled from 0.14 ± 0.07 μg ammonium-N g^{-1} *dw* (IG) to 0.28 ± 0.12 μg ammonium-N g^{-1} *dw* (TP) and finally reached 0.51 ± 0.22 μg ammonium-N g^{-1} *dw* (FS). Ammonium-N contents in the initial and final grassland (IG and CG) had the lowest values.

Different from the strong increase of nitrate-N and TNb the DOC content outlined its maximum in the transitional phase causing a decrease of the DOC/TNb ratio during the conversion. The nutrient stocks within the initial and the control grassland remained constant.

Table 1
Soil properties at the four sampling dates.

Sampling	Date	Soil temperature	Soil moisture	Soil pH	Samples
IG	08.07.2016	26.1 ± 0.6 °C	43.6 ± 2.6%	5.45 ± 0.18	$n = 24$
TP	04.11.2016	11.2 ± 0.9 °C	47.7 ± 3.4%	5.50 ± 0.13	$n = 24$
FS	08.06.2017	26.5 ± 0.2 °C	37.7 ± 2.0%	5.35 ± 0.08	$n = 16$
CG	01.08.2017	23.5 ± 1.2 °C	44.8 ± 2.1%	5.45 ± 0.20	$n = 16$

Soil properties at the four sampling dates (IG – initial grassland, TP – transitional phase, FS – final state and CG – control grassland). Table shows means and standard deviation for soil temperature, soil moisture, soil pH and the amount of samples taken.

3.3. Plant performance (TP – transitional phase)

Performance of intercropped *V. faba* after 70 days of vegetation showed a rather homogeneous distribution pattern of biomass development and pigments within the field experiment (Supplementary Table S2). The average plant height of *V. faba* plants was 51.6 ± 7.2 cm containing 1.05 ± 0.14 mg g^{-1} *fw* chlorophylls ($a + b$) and 0.26 ± 0.01 mg g^{-1} *fw* total carotenoids ($x + c$).

3.4. Bacterial community structure

3.4.1. Sequencing data

A total of 5.75 million raw reads were obtained from the sequencing platform of which 4.95 million raw reads (86.2% of total raw reads) remained after filtering and trimming. 4.60 million (80.1% of total raw reads) remained after denoising forward and reverse reads. After merging the reads 3.60 million (62.5% of total raw reads) and removal of chimeras 3.12 million (54.2% of total raw reads) reads remained. After removing ASVs not assigned to bacteria (NA and eukaryota), as well as those assigned to chloroplasts and mitochondria 3.11 million reads (54.0% of total raw reads) were remaining. 2.99 million reads (52.1% of total raw reads) were remaining after removing the negative controls and 2.80 million reads (48.7% of total raw reads) after filtering ASVs that were present in only one of the samples. The final ASV table contained on average 140,100 reads per sample with a minimum of 69,378 and a maximum of 320,927 reads counting for a sum of 8690 taxa. In total 27 phyla, 73 classes, 125 orders, 183 families, 314 genera and 24 species were unique.

The bacterial richness was not significantly different for the four sampling dates (Supplementary Fig. S3). Simpson's index of diversity (0.9990 ± 0.0003) indicated a highly diverse bacterial community structure on genus level throughout the entire experiment (Fig. 2). Although the diversity of FS was also very high (0.9986 ± 0.0002), it decreased significantly ($F = 5.745$ and $p < .007$) compared to the other sampling times (Supplementary Fig. S3).

Non-metric multidimensional scaling of beta-diversity revealed a good representation (stress-value of 0.108) of the sampling dates within two dimensions (Fig. 3). High similarity of the bacterial community structure could be seen for the initial grassland (IG) and the transitional phase (TP). Almost all of the individual samples clustered in the 95% confidence intervals of these sampling dates. The control grassland (CG) was most similar to the initial grassland (IG) and only slightly separated from the other sampling dates. Finally, FS clearly separated from the other sampling dates indicating a shift of the bacterial composition at the end of the experiment.

3.4.2. Soil bacterial communities

The most abundant phyla in our dataset were *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes* in decreasing order (Fig. 4). Members of these phyla accounted for 87.0 ± 6.9% of the total bacterial community structure. With an averaged relative abundance of 32.9 ± 2.0%, *Proteobacteria* was the predominant phylum during the entire experiment. However, also *Actinobacteria* (25.5 ± 1.9%), *Acidobacteria* (17.2 ± 1.9%), *Chloroflexi* (6.2 ± 0.4%), and *Bacteroidetes* (5.0 ± 0.8%) showed high abundances at the four sampling dates. *Proteobacteria* was the only of these phyla that changed significantly during the experiment having comparable values in IG, TP, and CG but higher abundances at the final state of the conversion (FS).

At the final state (FS) the higher abundance of the phylum *Proteobacteria* was correlated to the strong increase of members of the order *Betaproteobacteriales* (see Supplementary Table S3). Within samplings IG, TP, and CG averaged abundances of 6.6 ± 0.6% were observed, which more than doubled to 13.9 ± 2.3% at FS. Remarkably, the family *Burkholderiaceae* (*Betaproteobacteriales*) contributed strongly to this increase (Fig. 5). Starting with comparable abundances of 1.4 ± 0.3% (IG) and 1.7 ± 0.4% (TP) this family finally reached abundances of 10.4 ±

Table 2
Dissolved organic carbon (DOC), total nitrogen bound (TNb) and the ratio DOC/TNb in bulk soil.

	Unit	IG – initial grassland	TP – transitional phase	FS – final state	CG – control grassland
DOC	μg g^{-1} *dw*	15.3 ± 7.7[b]	30.9 ± 12.1[a]	12.5 ± 3.4[b]	20.5 ± 5.8[ab]
TNb	μg g^{-1} *dw*	5.2 ± 1.6[c]	15.2 ± 5.4[b]	45.3 ± 5.0[a]	8.1 ± 1.2[c]
DOC/TNb		3.0	2.0	0.3	2.5

DOC, TNb and the ratio DOC/TNb in bulk soil at the four sampling dates (IG, TP, FS and CG). The means and standard deviation for bulk soil expressed in μg g^{-1} *dw* of four biological replicates per plot and six plots per field ($n = 24$) are shown. Different letters (a, b, c) indicate significant differences ($p < .05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

2.5% (FS), representing the most abundant family within the whole experiment. With an averaged abundance of 4.6 ± 1.7% at FS, the genus *Massilia* (*Burkholderiaceae*) strongly contributed to this trend exhibiting the highest abundance of all genera within the entire experiment (see Supplementary Table S4). Interestingly, *Massilia* was not significantly present at the other sampling dates (IG, TP, and CG).

On order level members of *Rhizobiales* (*Proteobacteria*) were predominant during the experiment (see Supplementary Table S3). However, their abundance decreased significantly from 10.1 ± 1.0% (IG) to 7.8 ± 0.9 (TP) but finally increased again to 9.9 ± 0.3% (FS). With abundances of 6.8 ± 0.7% (IG) and 7.8 ± 0.3% (CG), the family *Xanthobacteraceae* was the most abundant family in the grassland plots contributing also to the high occurrence of *Rhizobiales* (Fig. 5). However, their abundance significantly decreased to 5.1 ± 0.7% (TP) but later reached the final state of 6.4 ± 2.8% (FS). *Pseudolabrys* was the most abundant genus within the family *Xanthobacteraceae* outlining highest abundances of 2.0 ± 0.3% in the grassland plots (Supplementary Table S4). Abundances during growth of *V. faba* and its incorporation were significantly lower (1.5 ± 0.2% (TP) and 1.6 ± 0.1% (FS)).

Myxococcales were observed as third most abundant order of the phylum *Proteobacteria*. Members of this order exhibited similar abundances of 4.6 ± 0.4% (IG, TP, and CG) which significantly decreased to 3.0 ± 0.3% at FS. Members of the genus *Haliangium* had the strongest influence on the decrease of *Myxococcales* at FS (see Supplementary Table S3). Similar trends were found for the fourth most abundant family, the *Nitrosomonadaceae* (*Proteobacteria*). This family outlined comparable values of 3.2 ± 0.2% within IG, TP, and CG but decreased significantly to 2.1 ± 0.1% at FS (Fig. 5).

Members of the order *Gaiellales*, which belong to the phylum *Actinobacteria*, showed similar abundances of 7.0 ± 0.6% (IG and TP) at the beginning of the experiment (see Supplementary Table S3). Interestingly, their abundances increased significantly to 9.5 ± 1.7% at FS. The most abundant genus, the *Gaiella*, outlined similar values of 1.8 ± 0.3% (IG) and 1.9 ± 0.5 (TP) at the beginning of the experiment. However, a significant increase after incorporation of the leguminous plant residues to 2.9 ± 0.9% (FS) was observed (see Supplementary Table S4). Similar trends on genus level were observed for *Lysobacter* (*Proteobacteria*), which was almost not present within IG, TP, and CG but significantly showed up in FS outlining abundances of 1.0 ± 0.3% (see Supplementary Table S4).

The most pronounced increase was observed for members of the order *Betaproteobacteriales*, its family *Burkholderiaceae* and therein its genus *Massilia* following the cultivation of *V. faba* and its subsequent incorporation (FS). Members of the order *Rhizobiales* and its family *Xanthobacteraceae* were found to be predominant during the entire experiment.

4. Discussion

The present study shows the successful transformation of a former marginal grassland (IG) to arable land (FS) via a transitional nitrogen fixing phase (TP).

Already in the beginning of the experiment after ploughing and milling the initial grassland and during growth of *V. faba* (TP) a strong enrichment of nitrate-N and TNb was observed. Mineralization processes following the incorporation of the residual green of the initial grassland (Chen et al., 2014) dominated this increase in the transitional phase. The subsequent further increase at the final state of conversion was dominated by nitrogen fixation of the legume (Fan et al., 2006) and the later incorporation of the leguminous plant residues (Ordóñez-Fernández et al., 2018). In total, the combined effects led finally to the high amount of 50 μg nitrate-N g^{-1} *dw* (150 kg N/ha) which is already sufficient for future crop cultivation.

Unlike the strong increase of nitrate-N and TNb, the carbon content (DOC) outlined its maximum in the transitional phase (TP) after incorporation of the grass residues and later decreased after incorporation of the leguminous plant residues (FS). The later decrease of DOC might indicate the utilization of soil organic carbon for bacterial immobilization of nitrogen after incorporation of the leguminous crop residues (Reichel et al., 2018).

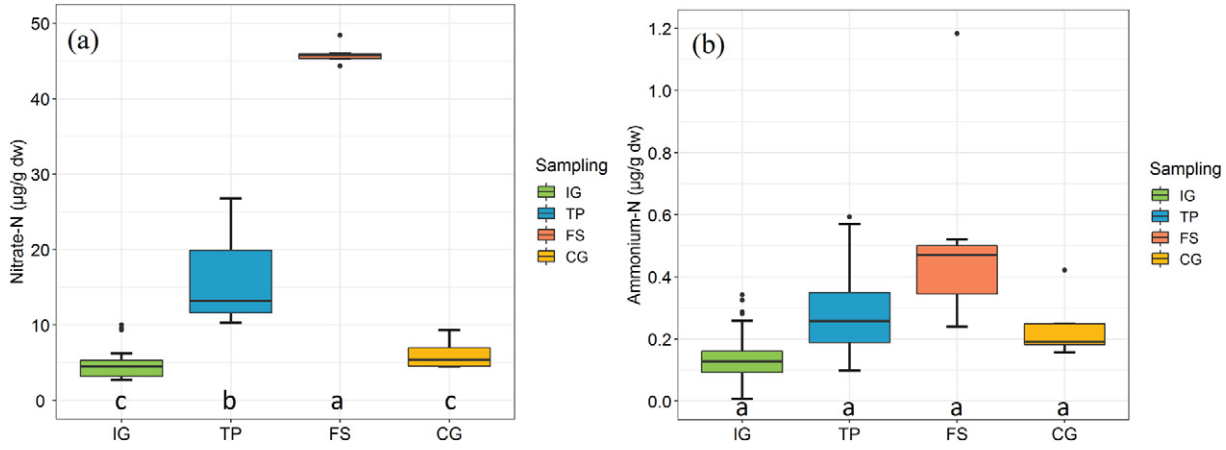

Fig. 1. (a) Content of nitrate-N and (b) ammonium-N in bulk soil expressed in μg N g^{-1} *dw* for four sampling dates (IG – initial grassland, TP - transitional phase, FS – final state and CG – control grassland). Data shown represent four biological replicates per plot and six plots per field ($n = 24$). Different letters (a, b, c) indicate significant differences ($p < .05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

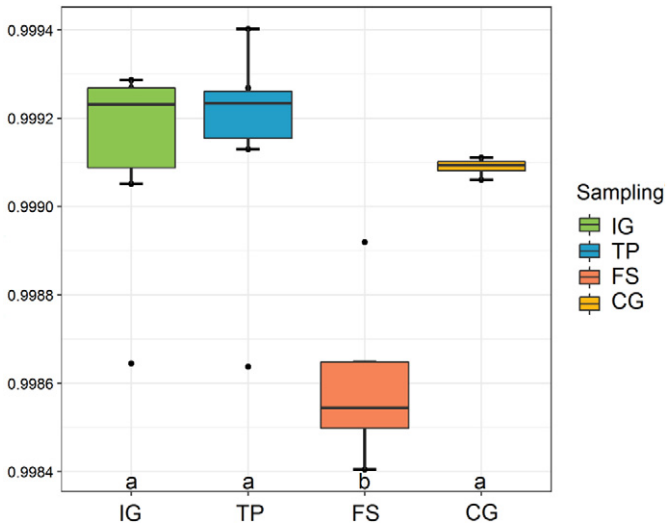

Fig. 2. Simpson's index of diversity for the four sampling dates (IG – initial grassland ($n = 6$), TP – transitional phase ($n = 6$), FS – final state ($n = 4$) and CG – control grassland ($n = 4$)). Different letters (a, b) indicate significant differences ($p < .05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

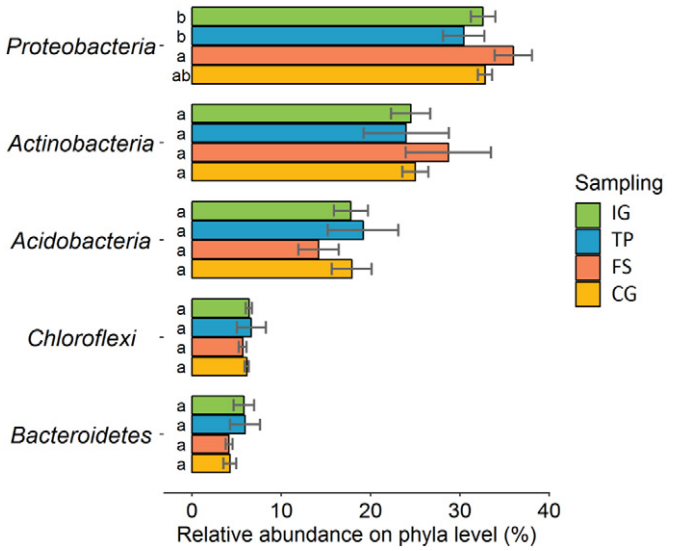

Fig. 4. Mean relative abundance (16S-Amplicon sequences) for the five most abundant phyla *Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi* and *Bacteroidetes* observed for four sampling dates (IG – initial grassland ($n = 6$), TP – transitional phase ($n = 6$), FS – final state ($n = 4$) and CG – control grassland ($n = 4$)) in descending order. Different letters (a and b) indicate significant differences ($p < .05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

The increasing amounts of TNb, as well as the decrease of DOC at the final state of the experiment, explained the decreasing trend of the DOC/TNb ratio. This ratio was with 3.0 highest in the initial grassland and only slightly lower in the control grassland. Interestingly, during the conversion the ratio decreased during growth of *V. faba* (TP) and reached its minimum after incorporation of the leguminous plant residues (FS) into the soil.

Soil properties of the initial and control grassland were comparable with respect to concentrations of nitrate-N, ammonium-N, DOC, TNb and its ratio DOC/TNb. It may hence be concluded that the strong increase of nitrate-N and the variations in DOC and the ratio DOC/TNb mainly depended on farm management and not on seasonal effects.

Plant performance and health of *V. faba* (Supplementary Table S2) observed on this field followed a homogenous pattern that is suitable for subsequent bacterial analysis. The prevailing pH was 5.4 ± 0.2 and thus optimal for root nodule formation (around 60 per plant) in *V. faba* (Torabian et al., 2019). The constant pH is one benefit of

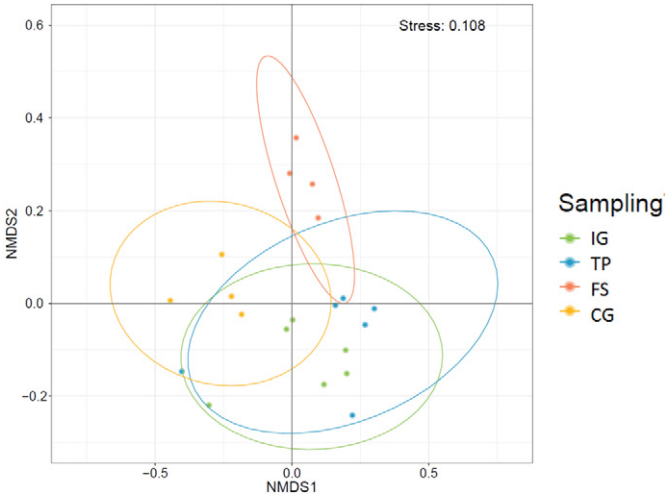

Fig. 3. Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities and ellipses on 95% confidence level for separation of bacterial community structure. Data shown on genus level according to the four different sampling dates (IG – initial grassland ($n = 6$), TP – transitional phase ($n = 6$), FS – final state ($n = 4$) and CG – control grassland ($n = 4$)). With 0.108 the stress value of the NMDS analysis revealed a good (<0.15) representation of the sampling dates within reduced dimensions ($k = 2$).

incorporating leguminous plant residues to enrich nutrient stocks within soil instead of using e.g. ammonium-based fertilizers since the increase of the net H^+ concentration after application of ammonium-based fertilizers leads to acidification of agricultural soils with negative effects on plants and organisms (Crews and Peoples, 2004). Different studies indicate pH as important factor for shaping bacterial community composition in grassland and agricultural soils (Kaiser et al., 2016; Wu et al., 2017). However, in our study effects of pH on bacterial community structure could be excluded because the pH remained constant independent of conversion state and spatial distribution within the field (see Table 1).

Simpson's index of diversity showed an extremely diverse bacterial community structure within the entire field experiment. The initial grassland (IG), the transitional phase (TP), and the control grassland (CG) had highest diversity during the conversion. In the final state (FS) diversity was significantly lower indicating a slight shift of bacterial composition to more dominant species when plant cover was lacking.

This trend was supported by a strong shift of beta-diversity toward the final state of the experiment. Furthermore, the NMDS analysis revealed high similarity for the initial grassland (IG) and the transitional phase (TP) indicating a quite stable bacterial community structure in the beginning of the experiment. However, although the control grassland (CG) outlined an overlap with the initial grassland (IG) and the transitional phase (TP), it slightly changed. Since soil nutrient stocks (nitrate-N, ammonium-N, DOC, and TNb) and environmental factors (soil temperature, soil moisture, and soil pH) of the initial and the control grassland remained quite stable this bacterial shift leads to the assumption that seasonal effects influenced the bacterial community structure in our grassland. Still, the control grassland differed significantly from the final state of the conversion (FS), indicating that the effects of the different conversion steps were much stronger than the seasonal effects. Drenovsky et al. (2010) and Xue et al. (2018) suggested accordingly that precipitation and elevation may have a weaker influence on shaping bacterial communities than soil properties and agricultural practices.

Phylogenetic lineage analyses based on 16S rRNA gene sequences showed highest abundances on phylum level for *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes*. Similar observations for dominant phyla in temperate grasslands were observed

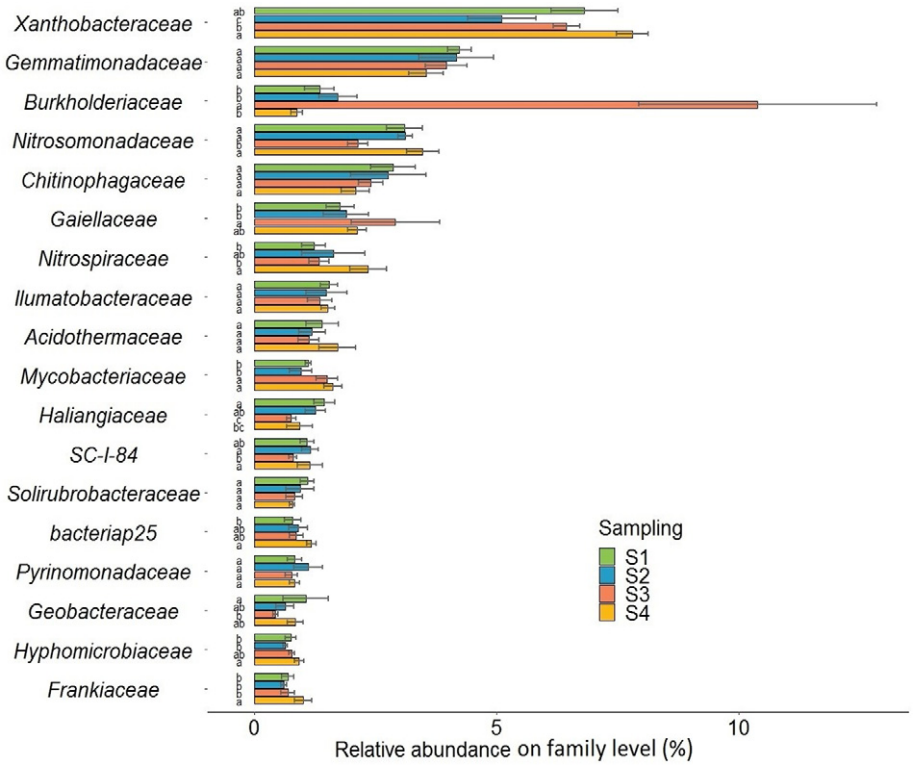

Fig. 5. Mean relative abundance (16S-Amplicon sequences) for the 18 most abundant families observed for four sampling dates (IG – initial grassland ($n = 6$), TP – transitional phase ($n = 6$), FS – final state ($n = 4$) and CG – control grassland ($n = 4$)) in descending order. Different letters (a, b, and c) indicate significant differences ($p < .05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

by Kaiser et al. (2016) and Delgado-Baquerizo et al. (2018). *Proteobacteria* were the predominant phylum during this study, significantly increasing at the final state after incorporation of the leguminous plant residues (FS). *Proteobacteria* are good indicators of crop residue degradability (Pascault et al., 2010) which explains their higher occurrence during decomposition of the incorporated legumes. Interestingly, no further significant changes on phylum level could be observed. It may hence be concluded, that grassland conversion and management practices did only slightly influence the bacterial community structure on phylum level. However, the previously described increase was mainly caused by increasing abundances of members of the order *Betaproteobacteriales* after incorporation of the leguminous plant residues (FS). This was mainly driven by the strong increase in abundance of the family *Burkholderiaceae* and its genus *Massilia*. Recent studies describe *Massilia*, formerly aligned to the family *Oxalobacteraceae*, as rhizosphere associated (Ofek et al., 2012) and plant-growth promoting rhizobacteria in leguminous plants (Xiao et al., 2017). Their high occurrence in bulk soil after incorporation of leguminous plant residues can be explained by bacterial decomposition of plant material and its release to the soil. Pascault et al. (2010) similarly reported strong increases of *Massilia* for early stages of decomposition of plant material. Other studies showed that several isolates of *Massilia* are able to reduce nitrate (Zhang et al., 2006; Bailey et al., 2014) which furthermore explains their occurrence in line with the highest nitrate-N content in bulk soil at the final state.

Similarly, increasing abundances of *Lysobacter* (*Proteobacteria*) after incorporation of *V. faba* may be explained by degradation processes of *V. faba* residues. Similar effects were also observed in tillage-residue management studies of Chávez-Romero et al. (2016). Interestingly, members of the genus *Lysobacter* have been described as promising candidates for biological control of plant diseases (Hayward et al., 2010), and increased abundances of *Lysobacter* might indicate improved soil quality (Wang et al., 2017).

Furthermore, members of the family *Gaiellaceae* strongly increased toward the final state of conversion. The family *Gaiellaceae* was found to be a good indicator of the carbon to nitrogen ratio (Hermans et al., 2017). The postulated negative correlation of its abundance to the DOC/TNb ratio could be supported by our findings, which showed an increasing amount of *Gaiellaceae* after incorporation of *V. faba* plant residues in line with the lowest DOC/TNb ratio.

Interestingly, members of the family *Nitrosomonadaceae* finally developed their lowest abundances after incorporation of plant residues. Similar results for decreasing abundances of the ammonia-oxidizing family after incorporation of some cover crops and organic fertilizer application have been reported (Fernandez et al., 2016). Similarly, members of the order *Myxococcales* outlined their lowest abundance in the final state. Herzog et al. (2015) showed lowered abundance of *Myxococcales* in line with lower carbon to nitrogen ratios. The most abundant phylotype within this order could be assigned to the genus *Haliangium*. High abundances of *Haliangium* in german grassland soils have also been shown by Kaiser et al. (2016).

All grassland plots were dominated by members of the order *Rhizobiales*. Kaiser et al. (2016) showed similar results for temperate grassland soils. Decreasing abundances in the transitional phase during growth of *V. faba* were linked to lower plant density and species diversity following the removal of the grass scar. Members of the family *Xanthobacteraceae* were most responsible for this decrease during *V. faba* growth and have been predominant in the grassland plots. *Pseudolabrys*, *Afipia*, and *Bradyrhizobium*, the three most abundant genera aligned to this family, have been identified to be diminished in the transitional phase. The subsequent increase at the final state may be due to the release of some members of *Rhizobiales* after decomposition of leguminous plant material. Denison and Kiers (2011) have reported similar effects on bulk soil for rhizobia (e.g. *Bradyrhizobium*) escaping from senescing nodules.

5. Conclusion

Overall, this study elucidates responses of soil bacteria after converting a temperate grassland to agricultural land via a transitional nitrogen-fixing phase. Results revealed a quite stable bacterial composition on phylum level, which was dominated by *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes*. Bacterial richness did not change during this short-term field trial. However, Simpson's index of diversity revealed a highly diverse bacterial community structure, which slightly decreased after the conversion. This change at the final state was supported by our beta-diversity analysis indicating changes due to the management practices. The study also revealed slight seasonal variations within the grassland plots. However, the change in the bacterial community structure was much more pronounced after converting the initial grassland to its final agricultural state. Strongest increase was observed for the family *Burkholderiaceae*, its genus *Massilia* as well as the genus *Lysobacter* after incorporation and decomposition of *V. faba* plants. The increase of the family *Gaiellaceae*, its genus *Gaiella* as well as the decrease of members of the order *Myxococcales* was linked to the decrease of the carbon to nitrogen ratio during the conversion. Furthermore, changes appearing already in the transitional phase were mainly induced by decreasing abundances of *Rhizobiales,* especially of its family *Xanthobacteraceae* caused by lower plant diversity. The strongly enriched nitrate-N, the lowered DOC/TNb ratio and effects occurring from decomposition processes were the main drivers of the community changes. Mineralization processes after grassland conversion, the nitrogen fixation of *V. faba* and its subsequent incorporation contributed to the strong mobilization of the nitrate-N pool in the final plots, ideal for further cropping of arable plants.

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Data availability

The nucleotide sequence data reported are available in the SRA database (NCBI) under the BioProject ID PRJNA471669.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.135494.

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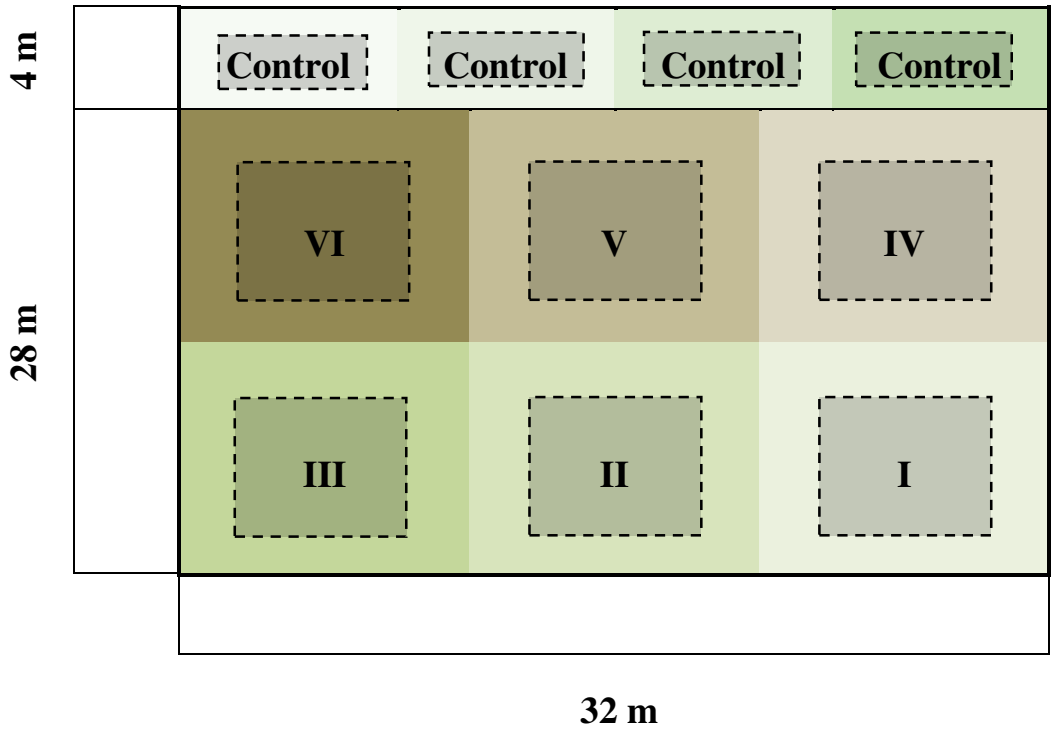
Appendix M1 (Supplements I)

Figures

Figure S1 Experimental field design (32 x 32 m) containing six subplots (I-VI) each 10.7 x 14 m and four control grassland plots (CG) each 8 x 4 m. The sampling region of each plot is delineated with a dashed line, in the center of the respective plots to avoid element exchange. The status of the field, at Martlhof (Bavaria, Germany), as well as management practices, sampling and analysis are described in the table. The field has been previously used as horse paddock (10 years), sheep pasture (3 years) and for free-range pigs (3 years). Mowing was done once a year, the farmer characterized the grassland as marginal and of low productivity that needed to be improved to provide revenue (Obermeier et al. 2020).



	IG Initial grassland		TP Transitional phase		FS Final state		CG Control grassland
Date	08.07.2016		04.11.2016		08.06.2017		01.08.2017
State	Grassland		*Vicia Faba* cover		Arable land (Bare soil)		Grassland
Management practices	IG	Tilling mulching, grass residues, milling, sowing Faba bean	TP	Incorporation of leguminous plant residues, tilling, ploughing, milling	FS	None	CG
Sampling	Soil and Biodiversity		Soil and Plant		Soil		Soil and Biodiversity
Analysis	16S, DOC, TNb, Nitrate, Ammonium, pH, Moisture, Temperature, Ellenberg		16S, DOC, TNb, Nitrate, Ammonium, pH, Moisture, Temperature, Pigments		16S, DOC, TNb, Nitrate, Ammonium, pH, Moisture, Temperature		16S, DOC, TNb, Nitrate, Ammonium, pH, Moisture, Temperature, Ellenberg

Figure S2 Mean daily precipitation (mm) and mean daily temperature (°C) at Tegernsee (Bavaria, Germany) from January 2016 to October 2017 (raw data received from the Bayerische Landesanstalt für Landwirtschaft describing the meteorological station in Rothenfeld, 2017). Sampling dates were IG – 08.07.2016 (green), TP – 04.11.2016 (blue), FS – 08.06.2017 (orange) and CG – 01.08.2017 (yellow).

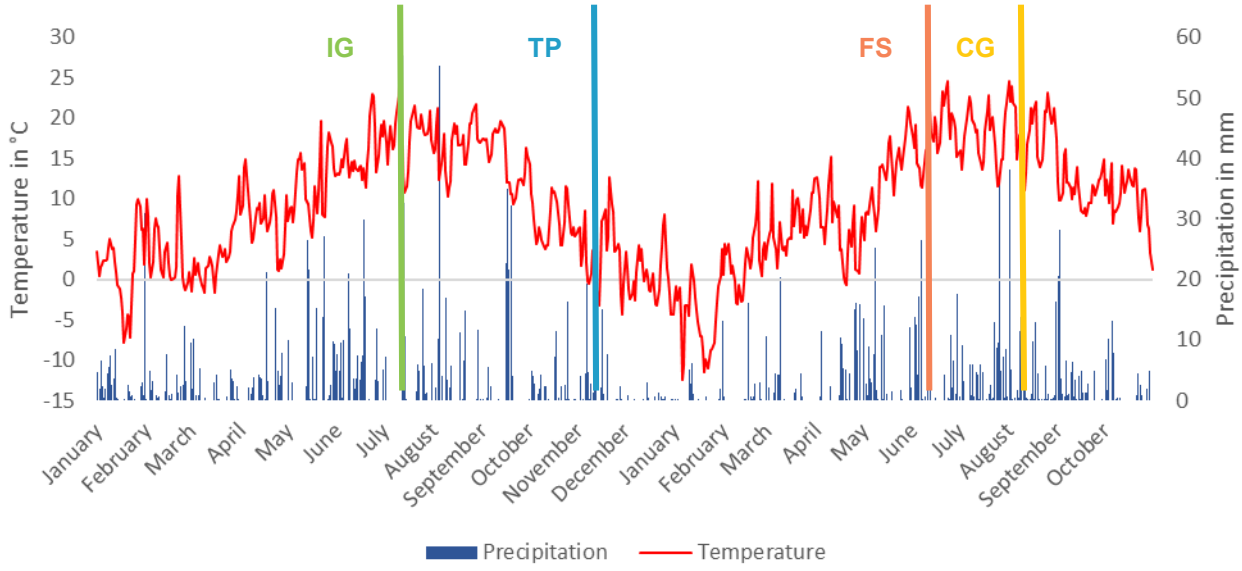
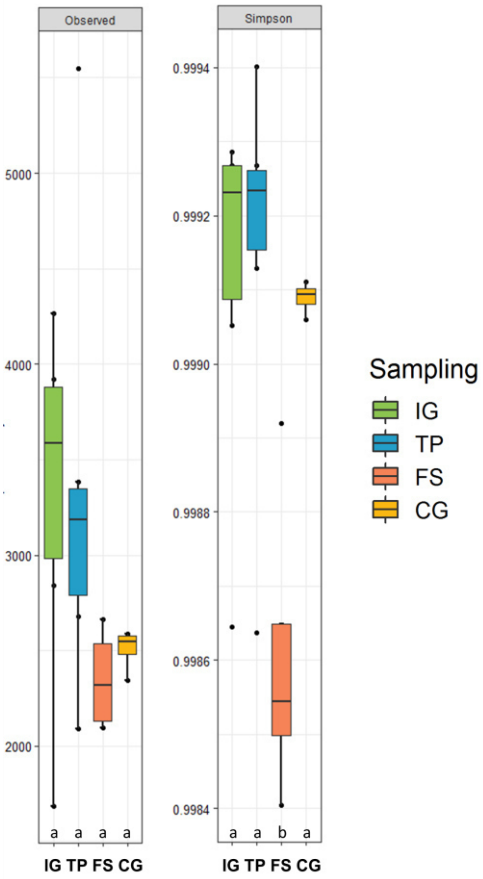


Figure S3 Different measures of α-diversity for the four sampling dates (IG – initial grassland, TP - transitional phase, FS – final state and CG – control grassland). Shown are boxplots as well as the data for Observed and Simpson diversity indices and the average over all sampling points (Mean) and its standard deviation (SD). Different letters (a, b) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test) (modified from Obermeier et al., 2020).



Sample	Sampling	Observed	Simpson
I1	IG	2841	0.99905
I2	IG	3920	0.99927
I3	IG	3408	0.99920
I4	IG	3766	0.99927
I5	IG	1685	0.99864
I6	IG	4267	0.99929
II1	TP	3125	0.99927
II2	TP	5550	0.99940
II3	TP	2089	0.99864
II4	TP	3242	0.99924
II5	TP	3384	0.99923
II6	TP	2677	0.99913
III26	FS	2493	0.99840
III28	FS	2141	0.99856
III30	FS	2663	0.99853
III32	FS	2097	0.99892
IV49	CG	2523	0.99910
IV50	CG	2571	0.99911
IV51	CG	2587	0.99906
IV52	CG	2345	0.99909
Mean	All	2967	0.99902
SD	All	881	0.00029

Tables

Table S1 Basic inventory following the guideline of Ellenberg (1992) for the initial and control state of the temperate grassland site in southern Germany (Tegernsee, Bavaria). Table shows species names, scientific names and values indicating the levels of individuality (I), sociability (S) and numbers for light (L), temperature (T), continental (K), moisture (F), soil acidity and lime content (R), nitrogen (N) and salt (S) (Obermeier et al., 2020).

Species name	**Scientific name**	**I** (1-5)	**S** (1-5)	**L** (1-9)	**T** (1-9)	**K** (1-9)	**F** (1-12)	**R** (1-9)	**N** (1-9)	**S** (0-9)
White clover	*Trifolium repens* L.	2	3	8	-	-	5	6	6	1
Red clover	*Trifolium pratense* L.	2	3	7	-	3	5	-	-	0
Ribwort plantain	*Plantago lanceolata* L.	2	2	6	-	-	5	-	-	0
Creeping buttercup	*Ranunculus repens* L.	3	4	6	-	-	7	-	7	1
Common sorrel	*Rumex acetosa* L.	4	4	4	6	2	8	7	7	0
Veronica	*Veronica officinalis* L.	1	1	6	-	-	5	-	-	0
Cow parsley	*Anthriscus sylvestris* (L.) Hoffm.	1	1	7	-	5	5	-	8	0
Cat grass	*Dactylis glomerata* L.	3	4	7	-	3	5	-	6	0
Common dandelion	*Taraxacum officinale* (L.) Weber ex F.H.Wigg.	3	1	7	-	-	5	-	8	1
Smooth meadow grass	*Poa pratensis* L.	2	2	6	-	-	5	-	6	0
Yellow oatgrass	*Trisetum flavescens* (L.) P.Beauv.	3	4	7	-	5	-	-	5	0
Meadow foxtail	*Alopecurus pratensis* L.	3	2	6	-	5	6	6	7	0
Himalayan balsam	*Impatiens glandulifera* Royle	2	3	5	7	2	8	7	7	0
Perennial ryegrass	*Lolium perenne* L.	1	1	8	6	3	5	7	7	0
Meadow fescue	*Festuca pratensis* Huds.	2	2	8	-	3	6	-	6	0
Broadleaf plantain	*Plantago major* L.	2	1	8	-	-	5	-	6	0
Common self-heal	*Prunella vulgaris* L.	2	1	7	-	3	5	7	-	0
Common wild oats	*Avena fatua* L.	1	1	6	6	6	5	7	-	0
Goosefoot	*Chenopodium album* L.	2	1	-	-	-	4	-	7	0

Table S2 Performance of intercropped *V. faba* plants after 70 days of vegetation for the six subplots in the transitional phase (TP). Table shows plant height in cm, content of chlorophylls ($a+b$) and total carotenoids ($x+c$) in mg g^{-1} *fw* (n = 6) as well as the standard deviation. The weight ratio $(a+b)/(x+c)$ indicates the greenness of plants (Lichtenthaler and Buschman 2001). Different letters (a, b, and c) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test) (Obermeier et al., 2020).

	Unit	I	II	III	IV	V	VI	Mean
Height	cm	53.7 ± 2.6^{ab}	56.3 ± 5.1^{a}	57.3 ± 0.6^{a}	56.3 ± 3.8^{a}	45.5 ± 1.9^{bc}	40.2 ± 2.6^{c}	51.6 ± 7.2
$a+b$	mg g^{-1} *fw*	0.95 ± 0.04^{b}	1.02 ± 0.04^{ab}	1.33 ± 0.09^{a}	1.12 ± 0.03^{ab}	1.02 ± 0.03^{ab}	0.87 ± 0.02^{b}	1.05 ± 0.14
x+c	mg g^{-1} *fw*	0.25 ± 0.06^{a}	0.26 ± 0.02^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.03^{a}	0.25 ± 0.01^{a}	0.26 ± 0.01
$(a+b)/(x+c)$		3.76^{b}	3.89^{b}	4.96^{a}	4.10^{ab}	3.71^{b}	3.46^{b}	3.98

Table S3 Relative abundances for the 9 most abundant observed orders (mean abundance given for the four sampling dates (IG – initial grassland (n = 6), TP – transitional phase (n = 6), FS – final state (n = 4) and CG – control grassland (n = 4)). Data shown are means and the respective standard deviation. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test) (Obermeier et al., 2020).

Phylum	Class	Order	IG	TP	FS	CG
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	$10.06 \pm 1.00\ \%^{b}$	$7.84 \pm 0.94\ \%^{c}$	$9.87 \pm 0.28\ \%^{b}$	$11.99 \pm 0.35\ \%^{a}$
Proteobacteria	*Gemmatimonadetes*	*Betaproteobacteriales*	$6.60 \pm 0.69\ \%^{b}$	$6.88 \pm 0.56\ \%^{b}$	$13.93 \pm 2.28\ \%^{a}$	$6.29 \pm 0.18\ \%^{b}$
Actinobacteria	*Thermoleophilia*	*Gaiellales*	$6.98 \pm 0.44\ \%^{b}$	$6.80 \pm 1.02\ \%^{b}$	$9.50 \pm 1.67\ \%^{a}$	$7.77 \pm 0.39\ \%^{ab}$
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	$4.90 \pm 0.47\ \%^{a}$	$4.77 \pm 0.45\ \%^{ab}$	$2.98 \pm 0.30\ \%^{c}$	$4.11 \pm 0.25\ \%^{b}$
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	$4.23 \pm 0.25\ \%^{a}$	$4.17 \pm 0.77\ \%^{a}$	$3.97 \pm 0.43\ \%^{a}$	$3.55 \pm 0.35\ \%^{a}$
Bacteroidetes	*Bacteroidia*	*Chitinophagales*	$3.78 \pm 0.59\ \%^{a}$	$3.82 \pm 1.02\ \%^{a}$	$3.05 \pm 0.28\ \%^{a}$	$3.07 \pm 0.44\ \%^{a}$
Actinobacteria	*Thermoleophilia*	*Solirubrobacterales*	$3.06 \pm 0.38\ \%^{a}$	$2.75 \pm 0.84\ \%^{a}$	$3.54 \pm 0.85\ \%^{a}$	$3.05 \pm 0.32\ \%^{a}$
Acidobacteria	*Acidobacteriia*	*Acidobacteriales*	$2.45 \pm 0.80\ \%^{a}$	$3.23 \pm 2.19\ \%^{a}$	$2.79 \pm 1.00\ \%^{a}$	$2.77 \pm 1.20\ \%^{a}$
Actinobacteria	*Actinobacteria*	*Frankiales*	$2.80 \pm 0.33\ \%^{ab}$	$2.61 \pm 0.30\ \%^{b}$	$2.60 \pm 0.44\ \%^{b}$	$3.33 \pm 0.15\ \%^{a}$

Table S4 Relative abundances for the 28 most abundant observed genera (mean abundance given for the four sampling dates (IG – initial grassland (n = 6), TP – transitional phase (n = 6), FS – final state (n = 4) and CG – control grassland (n = 4)). Data shown are means and the respective standard deviation. Additional genera with the most pronounced change during the sampling period (e.g. *Lysobacter*) are shown. Different letters (a, b, c, d) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

Phylum	Class	Order	Family	Genus	IG	TP	FS	CG
Actinobacteria	*Thermoleophilia*	*Gaiellales*	*Gaiellaceae*	*Gaiella*	1.78 ± 0.29 % ^{b}	1.90 ± 0.47 % ^{b}	2.91 ± 0.91 % ^{a}	2.13 ± 0.19 % ^{ab}
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Pseudolabrys*	2.06 ± 0.29 % ^{a}	1.52 ± 0.18 % ^{c}	1.60 ± 0.14 % ^{bc}	1.96 ± 0.15 % ^{ab}
Nitrospirae	*Nitrospira*	*Nitrospirales*	*Nitrospiraceae*	*Nitrospira*	1.23 ± 0.24 % ^{b}	1.64 ± 0.66 % ^{ab}	1.34 ± 0.21 % ^{b}	2.35 ± 0.38 % ^{a}
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*	1.50 ± 0.20 % ^{b}	1.24 ± 0.29 % ^{bc}	2.06 ± 0.47 % ^{a}	0.96 ± 0.07 % ^{c}
Actinobacteria	*Actinobacteria*	*Frankiales*	*Acidothermaceae*	*Acidothermus*	1.40 ± 0.34 % ^{a}	1.20 ± 0.28 % ^{a}	1.13 ± 0.22 % ^{a}	1.72 ± 0.38 % ^{a}
Actinobacteria	*Actinobacteria*	*Corynebacteriales*	*Mycobacteriaceae*	*Mycobacterium*	1.12 ± 0.06 % ^{b}	0.96 ± 0.24 % ^{b}	1.51 ± 0.22 % ^{a}	1.63 ± 0.18 % ^{a}
Proteobacteria	*Gammaproteobacteria*	*Betaproteobacteriales*	*Burkholderiaceae*	*Massilia*	0.02 ± 0.05 % ^{b}	0.12 ± 0.13 % ^{b}	4.64 ± 1.66 % ^{a}	0.01 ± 0.02 % ^{b}
Proteobacteria	*Deltaproteobacteria*	*Myxococcales*	*Haliangiaceae*	*Haliangium*	1.45 ± 0.21 % ^{a}	1.27 ± 0.21 % ^{ab}	0.77 ± 0.09 % ^{c}	0.94 ± 0.27 % ^{bc}
Proteobacteria	*Gammaproteobacteria*	*Betaproteobacteriales*	*Nitrosomonadaceae*	Ellin6067	1.12 ± 0.13 % ^{a}	0.99 ± 0.24 % ^{ab}	0.8 ± 0.13 % ^{b}	0.87 ± 0.06 % ^{ab}
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Afipia*	0.8 ± 0.08 % ^{b}	0.63 ± 0.1 % ^{b}	1.09 ± 0.15 % ^{a}	1.19 ± 0.13 % ^{a}
Acidobacteria	*Blastocatellia(Subgroup4)*	*Pyrinomonadales*	*Pyrinomonadaceae*	RB41	0.84 ± 0.15 % ^{a}	1.12 ± 0.3 % ^{a}	0.77 ± 0.12 % ^{a}	0.84 ± 0.11 % ^{a}
Proteobacteria	*Gammaproteobacteria*	*Betaproteobacteriales*	*Nitrosomonadaceae*	MND1	0.82 ± 0.14 % ^{b}	0.97 ± 0.19 % ^{ab}	0.47 ± 0.08 % ^{c}	1.13 ± 0.15 % ^{a}
Actinobacteria	*Actinobacteria*	*Propionibacteriales*	*Nocardioidaceae*	*Nocardioides*	0.60 ± 0.25 % ^{b}	0.85 ± 0.4 % ^{ab}	1.17 ± 0.29 % ^{a}	0.40 ± 0.13 % ^{b}
Actinobacteria	*Actinobacteria*	*Frankiales*	*Frankiaceae*	*Jatrophihabitans*	0.70 ± 0.12 % ^{b}	0.62 ± 0.06 % ^{b}	0.70 ± 0.14 % ^{b}	1.01 ± 0.19 % ^{a}
Proteobacteria	*Deltaproteobacteria*	*Desulfuromonadales*	*Geobacteraceae*	*Geobacter*	1.07 ± 0.47 % ^{a}	0.64 ± 0.18 % ^{ab}	0.44 ± 0.05 % ^{b}	0.85 ± 0.16 % ^{ab}
Firmicutes	*Bacilli*	*Bacillales*	*Bacillaceae*	*Bacillus*	0.78 ± 0.32 % ^{a}	0.59 ± 0.22 % ^{a}	0.82 ± 0.22 % ^{a}	0.8 ± 0.22 % ^{a}
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Rhodomicrobiaceae*	*Rhodomicrobium*	0.66 ± 0.14 % ^{ab}	0.5 ± 0.13 % ^{b}	0.81 ± 0.02 % ^{a}	0.78 ± 0.24 % ^{a}
Proteobacteria	*Gammaproteobacteria*	*Gammaproteobacteria*	*Unknown_Family*	*Acidibacter*	0.68 ± 0.17 % ^{a}	0.77 ± 0.18 % ^{a}	0.51 ± 0.11 % ^{a}	0.54 ± 0.15 % ^{a}
Proteobacteria	*Gammaproteobacteria*	*Betaproteobacteriales*	*Nitrosomonadaceae*	GOUTA6	0.59 ± 0.26 % ^{a}	0.68 ± 0.19 % ^{a}	0.38 ± 0.1 % ^{a}	0.75 ± 0.29 % ^{a}
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	*Pedomicrobium*	0.55 ± 0.06 % ^{b}	0.48 ± 0.04 % ^{b}	0.55 ± 0.03 % ^{b}	0.71 ± 0.11 % ^{a}
Proteobacteria	*Alphaproteobacteria*	*Rhodospirillales*	*Rhodopirillaceae*	*Defluviicoccus*	0.57 ± 0.07 % ^{a}	0.46 ± 0.19 % ^{a}	0.54 ± 0.19 % ^{a}	0.55 ± 0.21 % ^{a}
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Methyloligellaceae*	*Methyloceanibacter*	0.31 ± 0.05 % ^{c}	0.25 ± 0.04 % ^{c}	0.59 ± 0.04 % ^{b}	0.8 ± 0.05 % ^{a}
Proteobacteria	*Alphaproteobacteria*	*Rhizobiales*	*Xanthobacteraceae*	*Bradyrhizobium*	0.44 ± 0.04 % ^{ab}	0.38 ± 0.1 % ^{b}	0.48 ± 0.11 % ^{ab}	0.58 ± 0.11 % ^{a}
Bacteroidetes	*Bacteroidia*	*Chitinophagales*	*Chitinophagaceae*	*Ferruginibacter*	0.67 ± 0.20 % ^{a}	0.58 ± 0.24 % ^{ab}	0.33 ± 0.06 % ^{b}	0.28 ± 0.08 % ^{b}
Proteobacteria	*Gammaproteobacteria*	*Betaproteobacteriales*	*Nitrosomonadaceae*	mle1-7	0.45 ± 0.09 % ^{ab}	0.38 ± 0.1 % ^{b}	0.41 ± 0.06 % ^{b}	0.59 ± 0.07 % ^{a}
Proteobacteria	*Alphaproteobacteria*	*Reyranellales*	*Reyranellaceae*	*Reyranella*	0.44 ± 0.04 % ^{ab}	0.35 ± 0.08 % ^{b}	0.47 ± 0.06 % ^{a}	0.53 ± 0.06 % ^{a}
Actinobacteria	*Actinobacteria*	*Propionibacteriales*	*Nocardioidaceae*	*Marmoricola*	0.33 ± 0.13 % ^{ab}	0.57 ± 0.25 % ^{a}	0.66 ± 0.16 % ^{a}	0.20 ± 0.10 % ^{b}
Actinobacteria	*Actinobacteria*	*Micrococcales*	*Micrococcaceae*	*Pseudarthrobacter*	0.23 ± 0.10 % ^{b}	0.35 ± 0.14 % ^{b}	0.95 ± 0.07 % ^{a}	0.16 ± 0.06 % ^{b}
Proteobacteria	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	*Lysobacter*	0.02 ± 0.03 % ^{b}	0.16 ± 0.05 % ^{b}	1.04 ± 0.25 % ^{a}	0.01 ± 0.01 % ^{b}

ii. Appendix M2 (Manuscript II)

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REGULAR ARTICLE

Changes of soil-rhizosphere microbiota after organic amendment application in a *Hordeum vulgare* L. short-term greenhouse experiment

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Abstract

Aims In order to counteract the enduring decreases in the quality of agricultural land, mechanistic studies for a more sustainable agricultural crop production were performed. They aimed to assess the effects of organic amendments in combination with mineral fertilizer on soil-rhizosphere microbiota and their influence on soil health and plant performance.

Methods In a short-term greenhouse experiment, the effects of pelletized spent mushroom substrate, with different combinations of biochar and mineral fertilizer, on agricultural soil and performance of *Hordeum vulgare* L were scrutinized. To evaluate improved soil quality, different soil biological and chemical properties, microbial activity, bacterial diversity and plant performance were assessed.

Results Plant performance increased across all fertilizer combinations. Bacterial β-diversity changed from the initial to the final sampling, pointing at a strong influence of plant development on the rhizosphere with increasing abundances of *Acidobacteria* and decreasing abundances of *Actinobacteria, Chloroflexi*, and *Bacteroidetes*. Microbial activity (FDA), potential enzyme activity and metabolic diversity of the microbial community (BIOLOG) were not affected by the amendments, whereas bacterial community structure changed on family level, indicating functional redundancy. Treatments containing biochar and the highest amount of mineral fertilizer (B_MF140) caused the strongest changes, which were most pronounced for the families *Xanthobacteraceae, Mycobacteriaceae,* and *Haliangiaceae*.

Conclusion Applying organic amendments improved plant performance and maintained soil health, contributing to more sustainable crop production. Nevertheless, long-term field studies are recommended to verify the findings of this short-term experiment.

Keywords Bacterial community structure · Soil extracellular enzyme activity · Biochar · Spent mushroom substrate · Organic amendments · Mineral fertilizer · Biological soil quality indices

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Abbreviations

ASV	Amplicon sequence variant
BSA	Bovine serum albumin
B	Biochar
Z	Carbon
Chl	Chlorophyll
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
dw	Dry weight
fw	Fresh weight
FACCE-JPI	Joint Programming Initiative on Agriculture, Food Security and Climate Change
FDR	False Discovery Rate
INTENSE	Intensify production, transform biomass to energy and novel goods and protect soils in Europe
JE Lange	Jakob Emanuel Lange
L	Linné
LAI	Leaf area index
MBC	Microbial biomass carbon
MBN	Microbial biomass nitrogen
MUG	β-glucosidase
MUN	β-N-acetylhexosaminidase
MUP	acid phosphatase
MF	Mineral fertilizer
N	Nitrogen
NA	Not assigned
NMDS	Non-metric multidimensional scaling
OECD	Organization for Economic Co-operation and Development
P	Pellets
PB10	Pellets +10% biochar
PB20	Pellets +20% biochar
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
SMS	Spent mushroom substrate
TDN	Total dissolved nitrogen
TNb	Total nitrogen bound
TPB	Total plant biomass
V	Version

Introduction

Since the beginning of the twentieth century, application of mineral fertilizers has expanded agricultural production and increased yields to feed a rapidly growing world population (Erisman et al. 2008). This agricultural intensification and the continuously increasing need of food, feed, fiber and byproducts exerts tremendous pressure on the Earth's soils and their functioning. Excessive use of mineral fertilizer has been proven to be detrimental for soil microbial biomass, soil habitat functioning, plant species diversity, plant and even human health (Geisseler and Scow 2014; Horrigan et al. 2002). To mitigate these negative effects and to make agriculture more sustainable, the application of organic amendments (OA) obtained via cascading, upgrading and recycling of bio-based products has found raising interest (SCAR-report 2015; Schröder et al. 2018). Its application can influence various physical and chemical soil properties such as nutrient availability, soil aeration, water holding capacity and moisture (Bonilla et al. 2012a; Haider et al. 2016). Moreover, biological properties can be affected as shown for the soil microbial community structure and changes in its quantity, diversity and activity (Albiach et al. 2000; Bonilla et al. 2012b; Schmid et al. 2017).

Organic amendments have been reported to induce various positive but also negative effects on soil health and plant performance (Gómez-Sagasti et al. 2018; Schröder et al. 2018). For instance, application of residues from industrialized mushroom production (spent mushroom substrates; SMS), containing a high proportion of slowly decomposable lignocellulose (Hanafi et al. 2018), has proven positive effects on soil structure, microbial abundance and plant yield (Alvarez-Martín et al. 2016; Meng et al. 2018; Zhang et al. 2012). Additionally, biochar obtained from pyrolysis of organic wastes shows high potential in improving soil water retention, regulating the soil nitrogen cycle and decreasing nitrogen leaching (Haider et al. 2016; Liu et al. 2018; Ulyett et al. 2014). Its porous microstructure seems favorable for the colonization by microorganisms (Lehmann et al. 2011; Palansooriya et al. 2019). Furthermore, biochar incorporation into soil facilitates carbon sequestration and thereby contributes to the mitigation of climate change effects (Matovic 2011). The application of biochar during composting can be used to adjust the C/N ratio of the amendment which later influences soil microbial activity. Combination of different organic amendments with distinct features can improve overall amendment quality and reduce greenhouse gas emissions already during the composting process (Meng et al. 2018; Barthod et al. 2018).

Soil microorganisms are major drivers of the biochemical carbon and nitrogen cycle, thereby playing a

crucial role for soil health, its functioning and hence for crop and livestock health (Falkowski et al. 2008). The nitrogen cycle comprises dinitrogen fixation, assimilation into organic nitrogen, mineralization, nitrification subsequent denitrification and anaerobic ammonium oxidation (Kuypers et al. 2018). Its fluxes are defined by adsorption, mineralization, gaseous losses, plant uptake, leaching and microbial N immobilization (Murphy et al. 2000). The latter is driven by the composition of the microbial community and increases with higher C/N ratios of the organic amendments (Heijboer et al. 2016). The chemical composition of organic amendments affects the balance between plant N uptake and soil N retention and is therefore essential for plant growth. Amendments containing complex organic compounds (e.g. lignocellulose) trigger soil extracellular enzyme activity (EEA) to degrade these into lower molecular weight compounds like sugars, amino acids or ammonium (Burns and Dick 2002; Allison and Vitousek 2005). Soil microbial community composition and enzyme activity is hence pivotal for health and fertility of soils and thus to maintain crop performance.

Short-term greenhouse and long-term field experiments have recorded positive as well as negative effects of organic amendments in different cropping and soil systems (Prendergast-Miller et al. 2014; Schmid et al. 2017; Zhao et al. 2016). This highlights the importance of evaluating organic amendments in a holistic approach to reveal and understand the underlying mechanisms. Microbial indicators defining and monitoring soil quality and health are already abundant but the right choice and combination of the various indices is still under debate (Schloter et al. 2018).

For this study the barley cultivar Ella (*Hordeum vulgare* L. cv. Ella) was selected since it showed promising grain weight per plant and grain yield per plot (Surma et al. 2019). To evaluate the effects of organic amendments in combination with mineral fertilizer on soil-rhizosphere microbiota and performance of barley, different biological and chemical indices were used. Soil pH, mineral nitrogen, dissolved organic carbon/nitrogen, microbial biomass carbon/nitrogen, microbial activity and bacterial composition were analyzed to assess soil quality as well as plant morphology together with shoot and root carbon/nitrogen to describe plant performance. Potential soil microbial activity was determined photometrically, and bacterial diversity was analyzed by 16S amplicon sequencing. Working hypotheses were: (1) input of organic amendments maintains soil quality and improves plant performance, (2) input of organic amendments alone and in combination with mineral fertilizer changes microbial activity and bacterial community structure differently, (3) potential extracellular enzyme activity decreases after addition of mineral fertilizer (or increases by organic amendment addition), and (4) C/N ratios of the amendment combinations influence microbial N immobilization.

Material and methods

Soil and organic amendment characteristics

Soil for the greenhouse experiment was collected at Martlhof, in Ostin am Tegernsee (Bavaria, Germany) from the topsoil (0–20 cm) of a former study site for sustainable field management. A previous study (Obermeier et al. 2020) had focused on crop rotation, following the conversion of neglected grassland, using broad bean (*Vicia faba* L.) and fodder beet (*Beta vulgaris* L.). Average soil pH at the site ranged from 5.2–5.6 and its texture had been classified as clayey loam (28.2% sand, 43.1% silt and 28.8% clay). Solid organic amendments were applied to the pots as pellets (P, PB10 and PB20) and biochar granules (B) listed in Table 1. The pellets were produced by conventional composting and subsequent pelletizing of 50% spent mushroom substrate with 30% bio-rest from biogas production and 20% straw at a temperature of 59 °C and a compost humidity of 20–23% (pers. Comm. Prof. W. Szulc, Warsaw University of Life Sciences, Poland). Spent mushroom substrate was obtained after cultivation of *Agaricus bisporus* (JE Lange) Imbach. Additionally, 10 and 20% biochar was added to the pellets (PB10, PB20). Biochar had been produced from conifers and broadleaf trees through pyrolysis at 800 °C (Martlhof, Germany). It has a pH of 8.5 ± 0.1, organic

Table 1 Chemical composition of organic amendments. Abbreviations for the amendments used within the study, total nitrogen (N_{tot}) and total carbon (C_{tot}) in percentage and C/N ratio

	Organic amendment	N_{tot} [%]	C_{tot} [%]	C/N
P	Pellet	1.48	21.28	14
PB10	Pellet +10% Biochar	1.46	25.28	17
PB20	Pellet +20% Biochar	1.32	30.21	23
B	Biochar	0.23	71.19	310

matter content of 91.9 ± 5.0% and dry matter content of 81.8 ± 2.4% (pers. Comm. Prof. E. Maestri, University of Parma, Italy).

Experimental setup

Fertilization scheme

Thirteen combinations of organic amendments alone and in combination with mineral fertilizer were tested (Table S1). The initial soil contained 60 kg N ha^{-1} and was fertilized up to 200 kg N ha^{-1}. Organic amendments were applied to reach equal C_{tot} contents and a maximum of 140 kg N_{tot} ha^{-1}, except for treatment PB10N and PB20N. Here, 140 kg N_{tot} ha^{-1} were applied despite the higher C_{tot} content compared to the latter treatments. Calcium ammonium nitrate (CAN, Borealis L.A.T. GmbH, Linz, Austria) containing 27% nitrogen (1:1 nitrate and ammonium) was applied as mineral fertilizer in two conditions. First, to obtain maximum fertilization of 140 kg N_{tot} ha^{-1} (MF140) since due to C_{tot} equality, the treatments containing biochar were short in nitrogen, which was supplied by mineral fertilizer. Second, by adding 50 kg N_{tot} ha^{-1} (MF50) referring to a common fertilization practice according to local farmers. All calculations are in kilogram per hectare and refer to 30 cm soil depth and a bulk density of 1.5 t m^{-3}.

Setup and management

Plants were grown for 8 weeks until the majority reached the first nodal stadium (BBCH 31). Each of the 13 treatments and untreated controls were set up in four biological replicates, resulting in 56 independent pots. Standardized PVC DN 110 pipes (height 0.5 m) were sealed with plugs, drained with 0.7 kg crystal quartz sand (2–3.5 mm) and filled with 4.2 kg soil. To the upper 30 cm soil layer ground solid organic amendments were applied. Four spring barley seeds (*Hordeum vulgare* L. cv. Ella) per pot were sown (DANKO Hodowla Roślin Sp. z o.o., Kościan, Poland). When plants reached the two leaf stadium (BBCH 12), in the second week of the experiment, two seedlings were carefully removed and dissolved mineral fertilizer was applied. Pots were watered twice a week to obtain 60% water holding capacity. Throughout the experiment, pots were randomized and plant growth was supported by sodium-vapor lamps and a ceiling fan. The management and sampling scheme is given in Supplementary Fig. S1.

Sampling

Sampling was performed in the initial phase (week 1; initial) as well as in week 2 of the experiment, 4, 6 and finally during harvesting (week 8; final). Soil sampling to analyze soil properties and bacterial composition was performed at the initial ($n = 4$) and final sampling ($n = 56$) resulting in 60 independent soil samples. Five subsamples from each pot were taken in various depths, pooled, homogenized and subsequently sieved (2 mm) for later analysis. Due to the root architecture and extent of the rhizosphere the final soil samples comprised a mixture of rhizosphere and bulk soil and are thus defined as soil-rhizosphere. Soil samples (< 1 g) taken in week 2, 4 and 6 for extracellular enzyme activity measurements were collected from the upper 10 cm in the center of each pot to avoid disturbance of plant development. Soil material for chemical, enzyme and bacterial diversity analysis was stored at 4, −20 and − 80 °C, respectively. Shoots and roots of each pot were harvested separately. The phenological development stage of plants was determined weekly following the BBCH system according to Bleiholder et al. (2001). Chlorophyll content was measured during the second half of the experiment using a Dualex® Scientific Dx4.5 sensor (FORCE-A, Orsay, France).

Soil chemical analysis

Mineral nitrogen (N_{min}), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) were extracted from soil using 0.01 M $CaCl_2$ (1:4 *w*/*v*). Samples were shaken overhead using a horizontal shaker (Reax 2, Heidolph Instruments GmbH, Schwabach, Germany) at room temperature for 45 min and filtered through Whatman® filter type 595 1/2 (GE Healthcare, Buckinghamshire, UK). N_{min} as ammonium (NH_4^+), nitrate (NO_3^-) and total dissolved nitrogen (TDN) were analyzed photometrically following published methods (Obermeier et al. 2020) by continuous flow measurements using an autoanalyzer CFA-SAN Plus 5100 (Skalar Analytic, Erkelenz, Germany). DOC and DON were quantified using a DIMATOC®2000 (DIMATEC, Langenhagen, Germany). The gravimetric soil water content was determined after drying samples at 105 °C for 24 h. Soil pH was assessed in a 1:5 (*w*/*v*) dilution

with 0.01 M $CaCl_2$ following OECD guidelines (DIN ISO 10390 2005).

Plant analysis

Immediately after harvesting, barley leaves were scanned on an Epson Perfection 4180 Photo scanner (Epson®, Seiko, Japan) to determine the leaf area index (LAI). The green pixel content was analyzed with MATLAB® (The MathWorks® Inc., Natick, United States). The gravimetric water content of the plant material was determined after drying at 60 °C for 24 h. Total carbon (C_{tot}) and total nitrogen (N_{tot}) content of dried leaves and roots were determined after grinding them in a mixer mill (MM 400, Retsch®, Haan, Germany) and following combustion in an elemental analyzer (Euro EA, Eurovector Srl, Pavia, Italy).

Soil microbial analysis

Microbial biomass

Microbial biomass carbon (MBC) and nitrogen (MBN) were determined after chloroform fumigation of 5 g fresh soil with ethanol-free chloroform in a desiccator for 24 h (Brookes et al. 1985; Vance et al. 1987). Extraction and measurement of DOC and DON was performed as described above for soil chemical analysis (Joergensen 1995). MBC and MBN were calculated using k_{EC} 0.45 and k_{EN} 0.54 (Joergensen and Mueller 1996), respectively.

Microbial activity

Potential extracellular enzyme activities (EEA) were determined according to Pritsch et al. (2005). In short, methylumbelliferone (MU)-labeled substrates (Sigma-Aldrich, St. Louis, United States) in opaque 96-well plates (VWR™, Darmstadt, Germany) were used, and 50 µl soil suspension (400 mg soil in 40 mL sterile Milli-Q water mixed for 15 min and 22–25 µm filtered) was incubated in triplicates with 100 µl of the respective substrate saturation solution (see Pritsch et al. 2004). The substrate concentration and incubation time for each substrate/corresponding enzyme was determined in a pre-experiment as follows: MU-β-D-glucopyranoside/β-glucosidase (MUG, EC 3.2.1.21) 600 µM and 60 min, MU-N-acetyl-β-D-glucosaminide/β-N-acetylhexosaminidase (MUN, EC 3.2.1.52) 100 µM and 60 min and MU-phosphate/acid phosphatase (MUP, EC 3.1.3.2) 600 µM and 40 min. The enzyme reaction was stopped by adding 100 µl 1.25 M Tris buffer (pH > 10) and the plate was centrifuged for 3 min at 2420 rpm. Fluorescence was measured 20 min after reaction termination at excitation 365 nm and emission 450 nm wavelengths using a SpectraMax® Gemini™ EM microplate reader (Molecular Devices, Ismaning, Germany). A MU calibration curve (0, 1, 2, 3, 4, 5, 6 and 7 µM MU in Milli-Q water) and a soil quenching control (4 µM MU in soil suspension) were performed for each run. The maximum activity is expressed in picomol MU per gram dry soil per hour (pmol MU g^{-1} *dw* h^{-1}) according to German et al. (2011).

16S sequencing library preparation

DNA for sequencing was extracted from 500 mg soil using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, United States). Negative controls were introduced using empty extraction tubes. Quantification of extracted DNA was done by Qubit™ 4 Fluorometer and Qubit™ ds DNA Broad Range (BR) Assay Kit (Invitrogen™, Waltham, United States). Quality was assessed in a NanoDrop™ 1000 spectrometer (PeQlab Biotechnoloy, Erlangen, Germany). The 16S rRNA gene was amplified in the V1-V2 region using the primers S-D-Bact-0008-a-S-16 (5'-AGAGTTTG ATCMTGGC-'3) and S-D-Bact-0343-a-A-15 (5'-CTGCTGCCTYCCGTA-'3) (Klindworth et al. 2012). Therefore triplicated PCR reactions were performed using the NEBNext High-Fidelity 2X Master Mix (New England Biolabs, Ipswich, United States) and 5 pM of each primer, 3% bovine serum albumin (BSA), molecular grade water and 1 ng of extracted DNA. The PCRs were performed with the following program (see Obermeier et al. 2020): initial denaturation (98 °C, 30 s), followed by 28 cycles of denaturation (98 °C, 10 s), annealing (60 °C, 30 s) and elongation (72 °C, 30 s) and ended with a final elongation (72 °C, 5 min). PCR controls were performed under the same conditions and the quality of amplicons was visually assessed on a 1% agarose gel. Pooled samples of the three independent PCR reactions were purified using an Agencourt® AMPure® XP kit (Beckman Coulter Inc., Webster, United States) according to the manufacturers protocol for 96-well plates and with 1.3X the volume of the

sample for the beads. Quantification and quality of purified amplicons was assessed with a Fragment Analyser™ (Advanced Analytical Technologies GmbH, Heidelberg, Germany) using the DNF-473 Standard Sensitivity NGS Fragment Analysis Kit (1–6000 bp). The amplified and purified DNA of each sample was indexed using 10 ng and the Nextera® XT Index Kit v2 (Illumina Inc., San Diego, United States) with NEBNext High-Fidelity 2X Master Mix and molecular grade water resulting in 60 amplicon libraries. The indexing PCR program comprised an initial denaturation (98 °C, 30 s), followed by 8 cycles of denaturation (98 °C, 10 s), annealing (55 °C, 30 s) and elongation (72 °C, 30 s) and ended again with a final elongation (72 °C, 5 min). The indexed amplicons were checked and purified as described above. For sequencing on a MiSeq System with a read length of 2*300 bp (Illumina Inc., San Diego, United States) the amplicons were diluted to 4 nM, pooled equimolar and 11 pM DNA was loaded. The MiSeq Reagent Kit v3 (600 cycles) was used according to the manufacturer's protocol for paired-end sequencing and as a spike-in PhiX (Illumina Inc., San Diego, United States) was used as positive control.

Processing of the sequencing data

Primer and adapter removal of the de-multiplexed raw data obtained from the MiSeq system were performed with the AdapterRemoval software V. 2.1.7 (Lindgreen 2012), and amplicon sequencing errors were corrected using the model-based approach of the R package DADA2 V 1.8.0 (Callahan et al. 2016). Ten quality plots of forward and reverse reads were investigated, and accordingly quality filtering with a maximum expected error of three and a minimum read quality of two was performed. Forward and reverse reads were trimmed at 10 and 250 bp and 10 and 200 bp, respectively. Remaining contaminations by PhiX sequences were removed during quality filtering. Error modelling of reads and denoising of data was performed. This step comprised merging of paired-end reads and generation of a raw amplicon sequence variants (ASV) table and a chimera-cleaned sequence table. Finally, the ASVs were taxonomically annotated against the SILVA database V. 132 (Quast et al. 2013). Raw sequence data was imported into R V. 3.5.2 (R Core Team 2018). Filtering was performed by removing reads of negative controls, ASVs not assigned to bacteria and archaea as well as ASVs assigned to chloroplasts, mitochondria and ASVs singletons. The filtered sequence data was subsampled to the lowest read count over all samples using the rarefy function of the vegan package V. 2.5–4 (Oksanen et al. 2019). The raw nucleotide sequence data are available in the NCBI Sequence Read Archive (SRA) (Leinonen et al. 2010) under the BioProject accession number PRJNA540756.

16S sequencing data

Sequencing followed previously established procedures (Obermeier et al. 2020) and resulted in a total of 9.22 million raw reads of which 6.81 million (73.9% of total raw reads) remained after quality filtering, 6.33 million (68.6% of total raw reads) after denoising of forward and reverse reads, 5.15 million (55.8% of total raw reads) after merging and 5.10 million (55.3% of total raw reads) after chimera removal. The clearing of negative control reads, ASVs not assigned to bacteria or archaea (eukaryota and NA), ASVs assigned to chloroplasts or mitochondria and ASV singletons resulted in 5.04 million reads (54.6% of total raw reads). According to the rarefaction curves (Supplementary Fig. S5) all samples showed sufficient coverage of the bacterial community and subsampling resulted in 45,750 reads per sample. The final sequence data contained 2.74 million reads (29.8% of total raw reads) with 15,087 ASVs (97.1% of 15,531 raw ASVs). In total 33 phyla, 86 classes, 162 orders, 228 families, 463 genera and 61 species were aligned.

Statistical analysis

Statistical analysis of soil, plant and microbial data was conducted using R V. 3.5.2. One-way independent ANOVA ($p < 0.05$) and Kruskal-Wallis tests for not normal distributed data were performed using basic R functions to find differences between variants, following the procedure of Obermeier et al. (2020). The term variant includes the initial soil and the final soils comprising all treatments and controls. Normality of data and homogeneity of variance within each group was assessed by Shapiro-Wilk test and Bartlett test, respectively. Pearson and Spearman rank correlation tests for normal and not normally distributed data, respectively, were used to analyze shared variation between measurements to reveal correlations between soil, plant and microbial data. Shared variation was calculated as the

coefficient of determination (r^2) and is given in percentage (Field et al. 2012). Multiple comparisons of soil, plant and microbial data were performed using Tukey's honestly significant difference (HSD) post-hoc test in conjunction with a multivariate ANOVA ($p < 0.05$) using the agricolae package V. 1.3.0 (De Mendiburu 2014).

Subsampled sequencing data was analyzed using the phyloseq package V. 1.24.2 (McMurdie and Holmes 2013). To reveal effects of the different treatments on the bacterial composition bacterial α-diversity was calculated based on Shannon diversity index as well as Pielou's evenness index for species evenness. Bacterial β-diversity was analyzed by ordination using a multivariate principal coordinate analysis (PCoA) method based on Bray-Curtis dissimilarities. To confirm the results of the PCoA analysis a permutational multivariate analysis of variance (PERMANOVA) was performed using the vegan package. Relative abundances and standard deviations on different taxonomic levels were analyzed to indicate effects of different variants as well as the homogeneity of the biological replicates. In addition to the multiple comparisons performed with Tukey's post-hoc test, significant differences among treatments (FDR < 0.05) were checked using edgeR and DESeq2 analyses (Chong et al. 2020; Dhariwal et al. 2017). Associative relationships of plant and soil parameters to the most abundant bacterial families were identified using univariate ANOVA ($p < 0.01$).

Results

Plant performance

Aboveground plant biomass was different among treatments within the experiment ($F(13,42) = 3.60$, $p < .001$, $\omega = .61$). All treatments resulted in an increase in total plant biomass (TPB) compared to controls (Fig. 1). No significant difference was observed when OA had been applied alone compared to OA with MF (e.g. PB10 to PB10_MF140). Fertilization with pellets (P) alone and in combination with mineral fertilizer (P_MF50) resulted in the highest TPB significantly different to controls. In contrast, some treatments containing biochar (e.g. PB20_MF140, B_MF140, PB20_MF50 and B_MF50) resulted in a lower increase of TPB exhibiting a negative correlation with a small shared variation of 6% (Supplementary Fig. S2). In addition, a positive correlation with 87% shared variation for TPB and the leaf area index (LAI) as well as a negative correlation with 23% shared variation of TPB with nitrate-N were

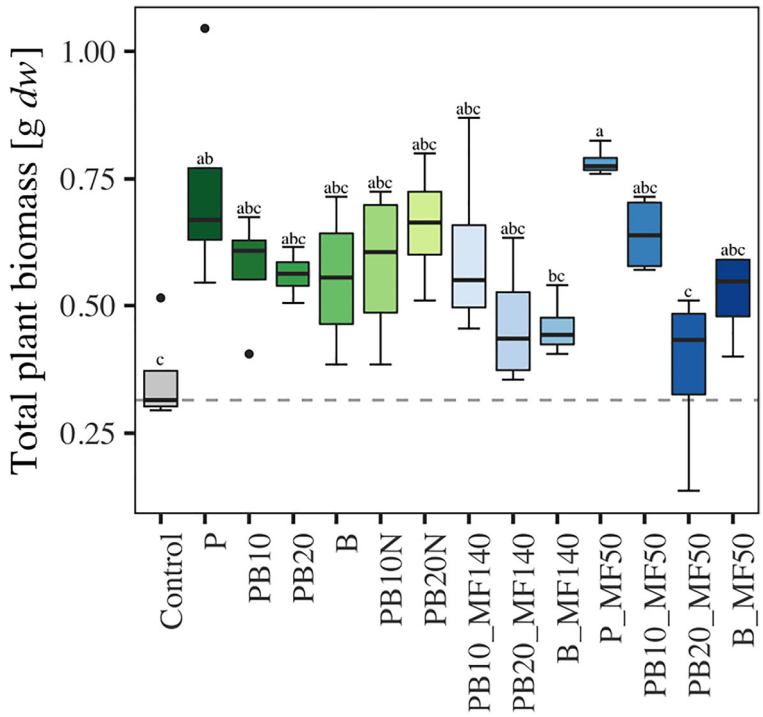

Fig. 1 Total plant biomass (TPB) in g *dw* given for the 14 treatments ($n = 8$). Gray dashed line marks median of the control. Different letters (a, b, and c) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test)

observed (Supplementary Fig. S2). Chlorophyll content, total carbon and nitrogen content of shoots and roots did not differ within the experiment (Supplementary Table S2).

Soil characteristics

Soil chemical analysis showed differences for nitrate-N ($F(14,45) = 12.71$, $p < .001$, $\omega = .86$), TDN ($F(14,45) = 10.03$, $p < .001$, $\omega = .82$) and DON ($chi^2(14) = 32.77$, $p = .003$). A significant decrease of up to 50% in nitrate-N between the initial soil and the final soil samples was observed (Fig. 2). In contrast, treatment B_MF140 led to an increase in nitrate-N compared to the initial soil and resulted in the only significant difference compared to controls. In general, treatments containing higher amounts of biochar and MF (PB20_MF140, B_MF140, PB20_MF50, and B_MF50) resulted in higher nitrate-N in the final soil mixture compared to treatments with only low amounts of biochar and MF (e.g. P, PB10, PB10_MF140, P_MF50, and PB10_MF50). A positive correlation with 31% shared variation for the amount of biochar applied to nitrate-N was found (Supplementary Fig. S2). DON and TDN showed a shared variation of 61% ($r = 0.78$, $p < .001$) and its ratios reflected the variability seen for nitrate-N but without significant decrease (Supplementary Fig. S3). Similar to nitrate-N, treatment B_MF140 resulted in a significant increase compared to both, control and initial soil. Other soil quality parameters, including pH_{CaCl2} with on average 5.1 ± 0.13, which was lowest in treatment B_MF140 (5.0 ± 0.08) as well as DOC and ammonium-N remained stable within this experiment (Supplementary Table S2).

Soil microbial activity

Microbial biomass carbon and nitrogen were on average 587.6 ± 85.6 μg g^{-1} *dw* and 62.1 ± 20.5 μg g^{-1} *dw*, respectively. Due to the high variability within replicates no significant difference between treatments, controls or initial soil was observed. With 10.3 ± 3.4 the microbial biomass C/N ratio remained constant within the experiment (Supplementary Table S2).

The potential overall microbial activity analyzed by FDA hydrolysis did not differ between treatments and controls. Carbon metabolization was different regarding average well color development (AWCD) between some treatments (highest for P_MF50 and lowest for P and PB20_MF140) but not compared to controls

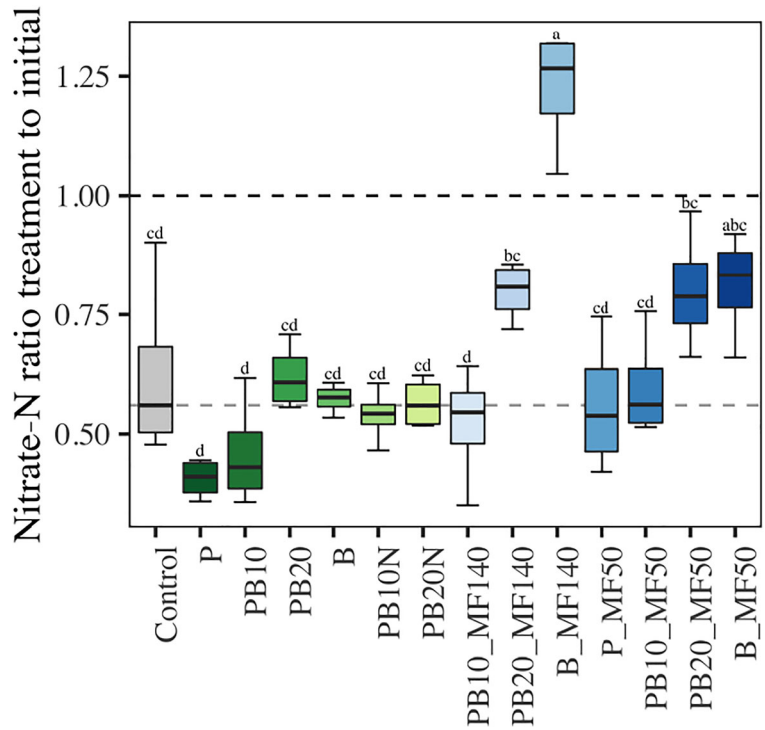

Fig. 2 Soil nitrate-N ratio of the 14 treatments ($n = 4$) compared to the mean of the initial soil. Black dashed line marks ratio of 1.0 and indicates no difference. Gray dashed line marks median of the control. Different letters (a, b, c, and d) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test)

(Supplementary Fig. S4). Carbon metabolic functional diversity and evenness, presented as Shannon and Pielou's evenness index, were not significantly different between treatments including controls (Supplementary Table S3).

The maximum potential activities of β-glucosidase, acid phosphatase and β-N-acetylhexosaminidase were not significantly different between treatments including controls within the four sampling time points (d0, d14, d28, and d42) (Supplementary Table S3). However, significant differences were observed across all treatments for the average maximum potential activity of the three enzymes regarding sampling time (Fig. 3). Highest activity was measured in week 4, 14 days (d14) after mineral fertilizer application and lowest activity in week 8 (d42).

Bacterial community structure

Diversity analysis

Bacterial α-diversity (Shannon) and species richness were not significantly different throughout the experiment (Supplementary Fig. S6). However, a significant effect was observed for Pielou's species evenness as shown in Fig. 4 ($F(14,45) = 3.66$, $p < .001$, $\omega = .62$). Highest evenness was observed for the initial soil and lowest for treatment B_MF140. Controls showed high variance and did not differ significantly from treatments and the initial soil. None of the diversity measurements revealed any correlation with soil and plant parameters.

The observed difference in evenness was also explained by bacterial β-diversity assessed with PCoA and sampling as major factor (Fig. 5). The factor sampling comprised the two groups initial and final, with the latter clustering the fourteen different treatments including the control. The factor was proven to be a significant determinant of bacterial community structure in a permutation test ($F(1,58) = 4.48$, $p < .001$).

Differences in relative abundance between samplings

The six most abundant phyla within the greenhouse experiment were *Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, Chloroflexi,* and *Bacteroidetes* in decreasing order (Fig. 6). Together

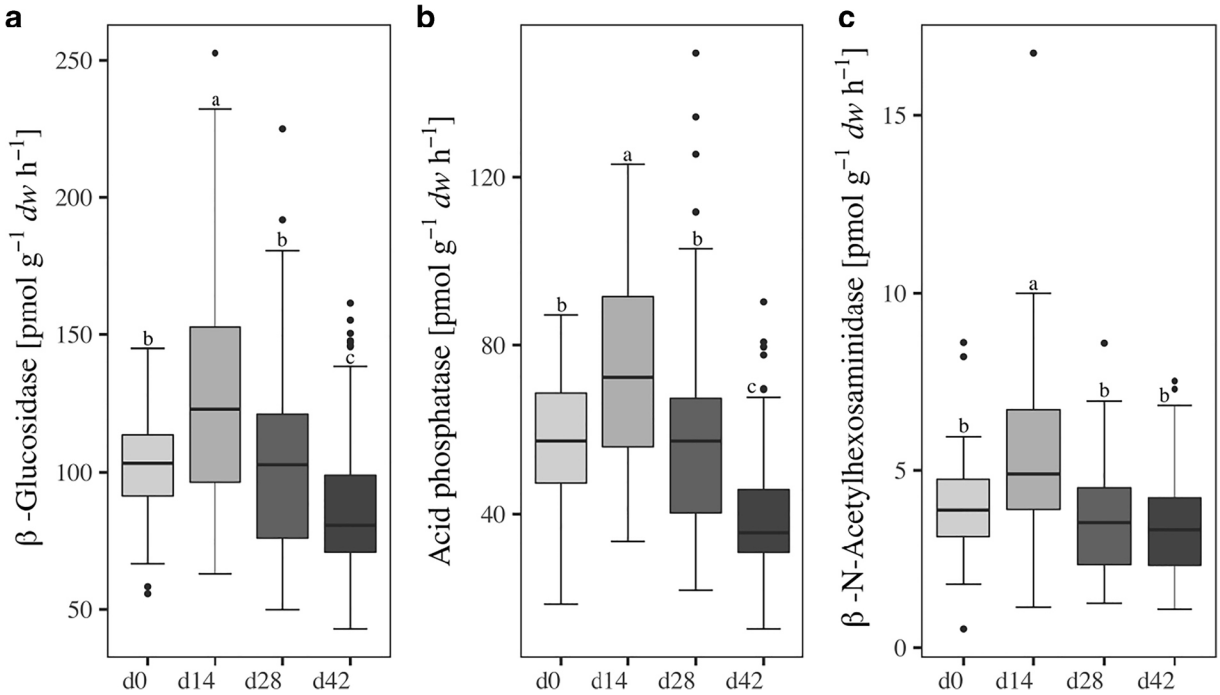

Fig. 3 Maximum potential activity of **a** β-glucosidase, **b** acid phosphatase and **c** β-N acetylhexosaminidase in pmol MU $g^{-1}dw\ h^{-1}$ on average ($n = 56$) for the different sampling times in days (d0, d14, d28, and d42). Different letters (a, b, and c) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test)

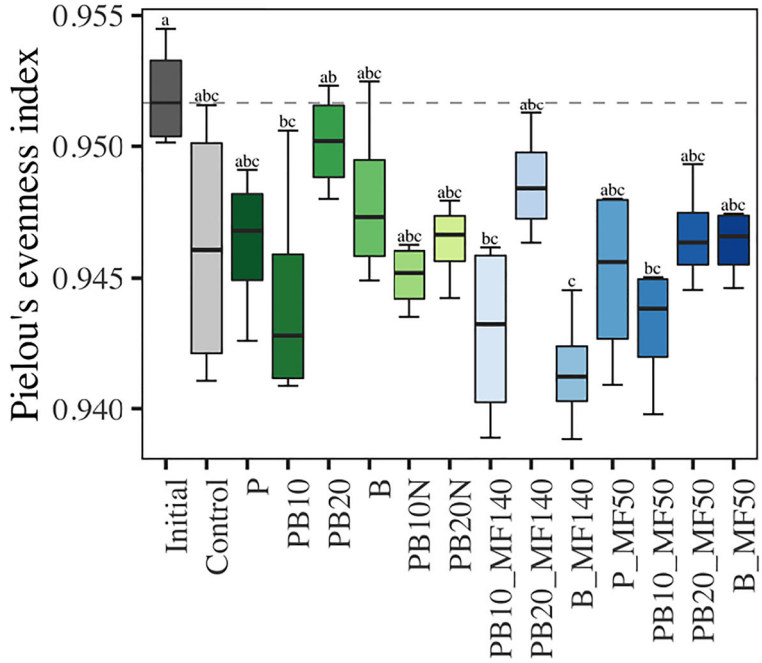

Fig. 4 Bacterial α-diversity within the experiment illustrated as Pielou's species evenness index calculated for the 15 variants ($n = 4$). Gray line marks median of the initial soil. Different letters (**a**, **b**, and **c**) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test)

these phyla accounted for 90.4 ± 9.1% of the total bacterial community structure. With an averaged relative abundance of 36.3 ± 2.2%, *Proteobacteria* was the predominant phylum within the experiment. However, also *Acidobacteria* (23.0 ± 2.2%), *Actinobacteria* (19.0 ± 2.5%), *Gemmatimonadetes* (6.0 ± 0.5%), *Chloroflexi* (3.1 ± 1.1%) and *Bacteroidetes* (3.1 ± 0.6%) showed high abundances for both sampling times and across treatments. Similar to the β-diversity analysis the most pronounced differences were found between initial and final sampling. This was mainly driven by the increase of *Acidobacteria* and the decreasing abundances of *Actinobacteria, Chloroflexi*, and *Bacteroidetes* in the final samples. No significant differences among treatments at the final state of the experiment for the six most abundant phyla were observed. Further analyses using

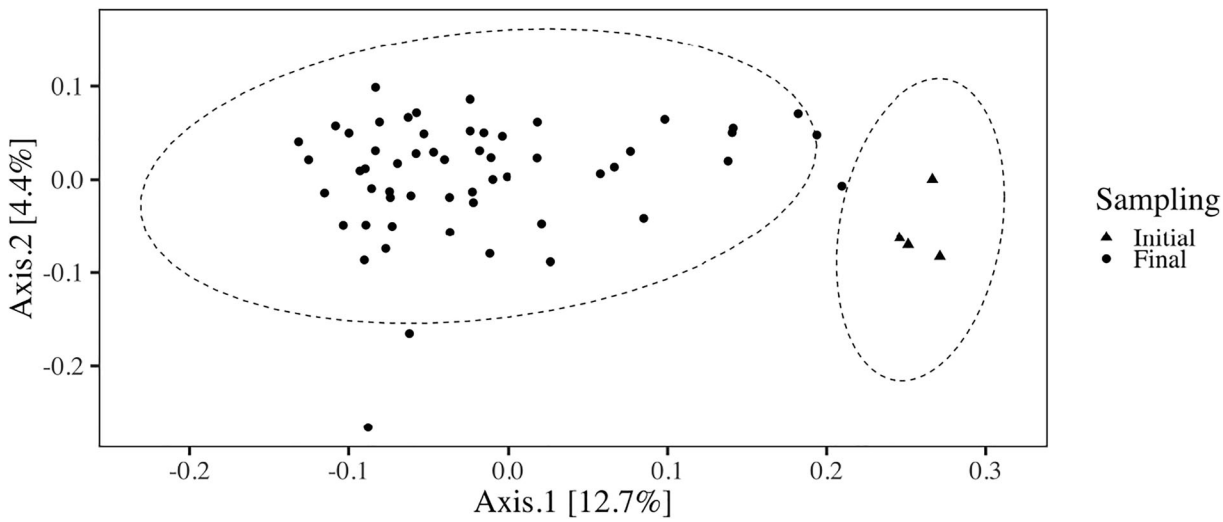

Fig. 5 Bacterial β-diversity presented with principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities. Shown are ellipses on 95% confidence level for separation of bacterial community structure according to the factor sampling with initial (triangles) and final (dots). Axis 1 and 2 account for 12.7 and 4.4% of the variation, respectively

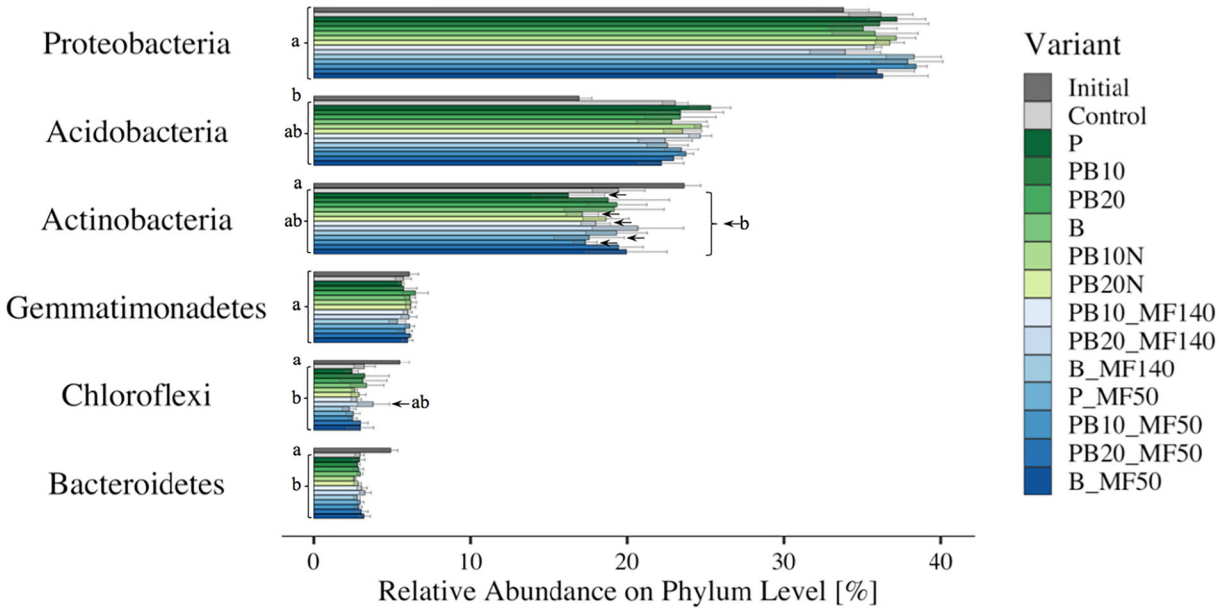

Fig. 6 Mean relative abundances for the six most abundant phyla observed for the 15 variants ($n = 4$) in percent. Different letters (**a** and **b**) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test)

edgeR and DESeq2 did also not reveal any significant differences among treatments (FDR < 0.05), even for rare phyla.

Although *Proteobacteria* did not show any change on phylum level, changes on class level could be observed (Supplementary Fig. S7). *Alphaproteobacteria,* the most abundant class, resulted in increasing and *Gammaproteobacteria,* the second most abundant class, in decreasing abundances at the final sampling. The increase of *Alphaproteobacteria* was mainly influenced by *Xanthobacteraceae,* the most abundant family of the experiment (Supplementary Table S4). The decrease of *Gammaproteobacteria* was mainly caused by a 23.7 ± 12.9% decrease of *Nitrosomonadaceae*, the third most abundant family (Fig. 7).

Besides *Nitrosomonadaceae,* the most pronounced decrease on family level compared to the initial soil was observed for *Chitinophagaceae* (30.6 ± 7.8%), *Nitrospiraceae* (27.0 ± 13.2%), *Xanthomonadaceae* (37.8 ± 14.8%), and *Burkholderiaceae* (57.7 ± 6.6%) (Supplementary Table S5). On the contrary, the most pronounced increase compared to initial was observed for *Xanthobacteraceae* (38.0 ± 15.1%), *Mycobacteriaceae* (57.6 ± 21.1%) and *Pyrinomonadaceae* (98.4 ± 37%). Abundances of *Gemmatimonadaceae* and *Gaiellaceae* remained almost constant within the experiment.

Differences in relative abundance between treatments

Although the most pronounced difference in bacterial abundance was observed between initial and final soils, also minor changes were seen between treatments at the final sampling. Especially treatment B_MF140 was striking, exhibiting highest relative abundances for *Xanthobacteraceae* (12.4 ± 1.0%) and *Mycobacteriaceae* (2.6 ± 0.3%) and lowest for *Haliangiaceae* (1.0 ± 0.1%) at the final sampling (Supplementary Table S4). On the contrary, treatment PB20_MF140 outlined lowest relative abundances for *Xanthobacteraceae* (9.6 ± 0.9%) and *Mycobacteriaceae* (1.9 ± 0.2%) across all treatments. Similar trends were shown for the ratio of the respective treatments compared to initial as visualized in Fig. 7. No significant differences across different fertilizer combinations (treatments) could be observed for *Gemmatimonadaceae*, *Nitrosomonadaceae*, *Solirubrobacterales (67–14)*, *Gaiellaceae*, *Chitinophagaceae*, *Nitrospiraceae*, *Pyrinomonadaceae*, and *Burkholderiaceae* at the final sampling (Supplementary Table S5). Additional in-depth analyses among treatments using edgeR including all families, revealed significant differentiation (FDR < 0.05) for ten families with only three of them being members of the 12 most abundant ones

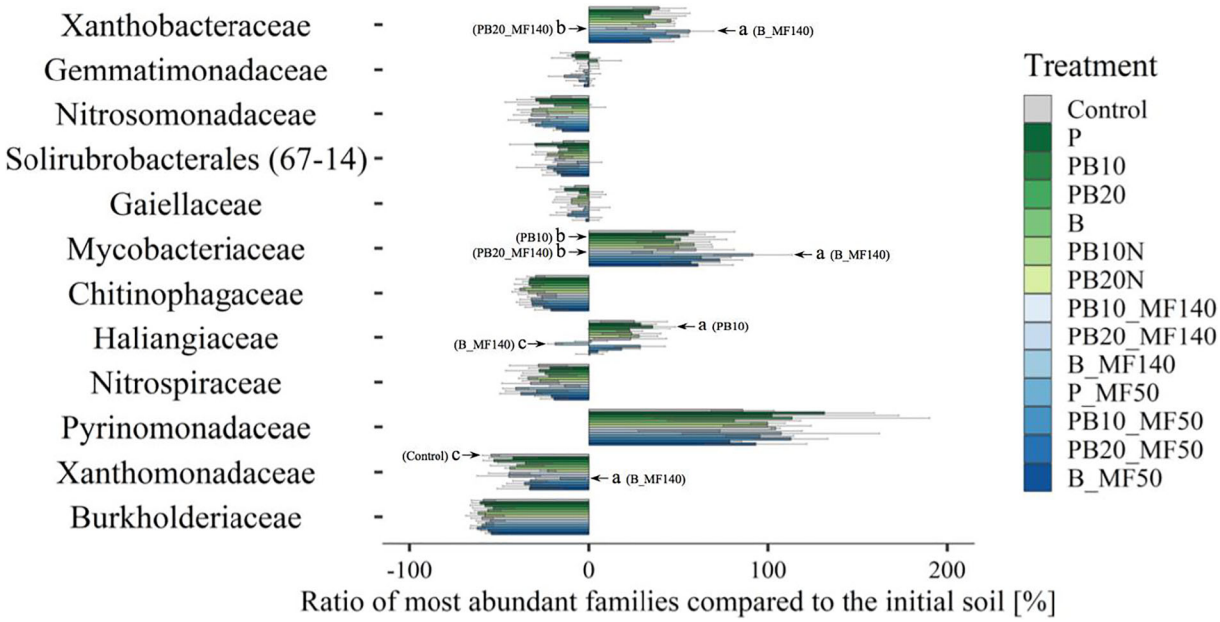

Fig. 7 Relative abundance ratio of the 12 most abundant families compared to the mean of the initial soil for the 14 different treatments ($n = 4$). Arrows and letters (**a**, **b** and **c**) indicate significant differences among the treatments ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test) for the highest and lowest abundances. Statistical data not included in the plot is given in Supplementary Table S5

(Supplementary Table S6). Similar to the pattern observed for relative abundance analysis and ratio plot (Fig. 7) the majority of families (7 out of 10) revealed the most pronounced difference for treatment B_MF140 compared to the other treatments.

Relationships of soil and plant parameters to the most abundant families at the final sampling

Highly significant associations ($p < 0.01$) of eight soil and plant parameters with the 12 most abundant families at the final sampling were observed (Fig. 8). The majority of families exhibited an associative relationship to microbial carbon (MBC) and nitrogen (MBN) at the final sampling time. Only *Gaiellaceae*, *Haliangiaceae*, and *Xanthomonadaceae* did not show any association to MBC and MBN. Nevertheless, *Gaiellaceae* showed a relationship to TDN and *Xanthomonadaceae* to nitrate-N. The family *Haliangiaceae* was associated not only to TDN and nitrate-N but also to the plant parameters TPB and LAI. Another association to those plant parameters was only found for unclassified bacteria *67–14* of the order *Solirubrobacterales*. Even though the maximum potential activity of *β*-glucosidase (MUG) and acid phosphatase (MUP) was weakest at final sampling (Fig. 3), an association to the families *Pyrinomonadaceae*, *Burkholderiaceae* and *Nitrosomonadaceae* (for MUP only) was found.

Discussion

Stable soil quality and intensified plant performance

Effects of organic amendments and mineral fertilizer on soil-rhizosphere microbiota are known to directly influence soil quality and plant health. Since soil quality cannot be measured directly, different fast responding chemical and biological indicators had to be assessed within this study. Soil total carbon and soil structure analyses were not performed within this pot study due to the short-term experimental design.

The most variable soil quality indicators were nitrate-N, TDN and DON. Decreases of nitrate-N in most of the treatments compared to the initial soil indicated nitrate uptake by the plant, which was also proven by the negative correlation of total plant biomass (TPB) to nitrate-N. Even though nitrate-N was reduced in soil by plant uptake, TDN and DON remained almost constant during the experiment, indicating a stable pool of

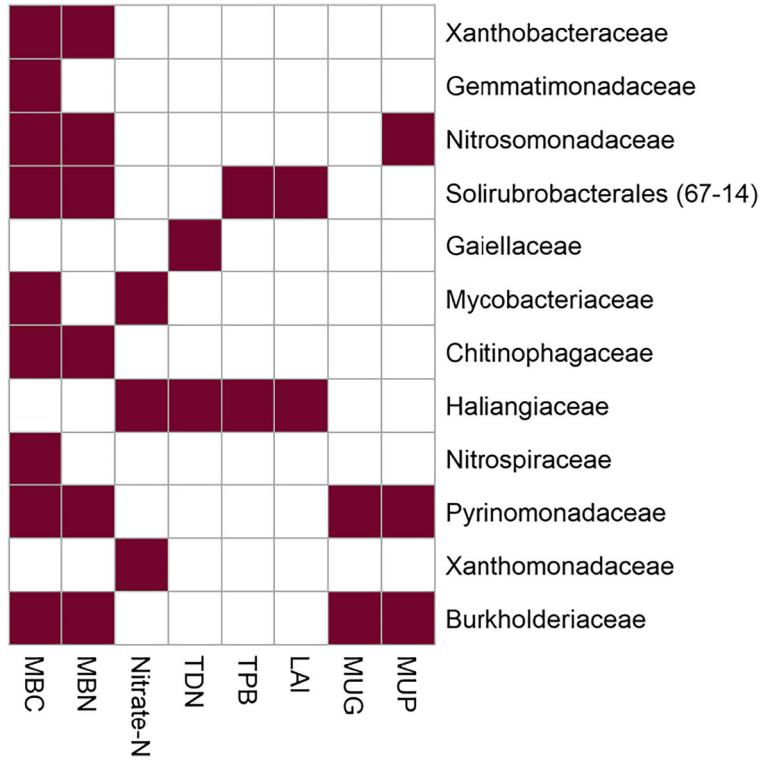

Fig. 8 Heatmap showing associations between the 12 most abundant families (decreasing order) to the 60 different soil and plant parameters taken at the final sampling. Associative relationships (darkred) with $p < 0.01$ (univariate ANOVA) are considered significant and those with $p > 0.01$ are considered insignificant (white). Shown are only those soil and plant parameters sharing at least two significant p-values with the most abundant families

organic nitrogen. The unchanged DOC content within the microcosms also reflected this stability of the organic pool. In addition, the unaffected content of MBN in the soil-rhizosphere environment indicated that the C/N ratio of the OAs did not induce microbial N immobilization.

The significant increase of nitrogen observed for treatment B_MF140 is likely due to application of mineral fertilizer, which was almost 3-times as high as for all other MF treatments (Supplementary Table S1). Nonetheless, a trend evolved towards higher nitrate-N contents remaining in soils amended with higher amounts of biochar and MF compared to treatments containing no or only low amounts of biochar and MF (Supplementary Fig. S2). This trend might be explained by the N_{min} retention capacity of biochar, described by Prendergast-Miller et al. (2014) who also observed higher nitrate-N after cultivating *H. vulgare* plants in biochar amended soils. However, the effects observed in this short-term experiment are only weak, since the initial soil (see controls) contained sufficient nitrate-N for plant growth.

Best plant performance was observed in treatments P and P_MF50 and was therefore independent of mineral fertilizer application. Nutrients provided by the pellets alone were sufficient to intensify plant performance and maintain good soil quality. No beneficial effects of combining organic amendment with mineral fertilizer on plant performance, different from Zhao et al. (2016), were observed within this study. However, a slight trend towards decreasing TPB alongside with an increase of biochar could be observed (Supplementary Fig. S2). This is in accordance with Liu et al. (2018) who outlined the importance of the correct dosage of biochar for positive effects on plant performance.

Dynamics of microbial activity

The influence of treatments/amendments on soil quality was further assessed by measuring soil enzyme activities, since higher activities often seem to be linked to healthier soils and accelerated nutrient transformation (Caldwell 2005). Many studies report on increasing enzyme activities after organic amendment application, with organic matter input as driver of microbial activity (Li et al. 2018; Zhao et al. 2016; Zhou et al. 2019). This increase was not observed in the present study, where overall microbial activity (FDA), metabolic diversity of

the microbial community (BIOLOG) and the three potential EEAs did not differ between treatments and controls, regardless of mineral fertilizer application. Hence, no increase of enzyme activity was observed in soils amended with a combination of OA and MF, which is different from observations of Zhao et al. (2016). However, these results must be interpreted carefully, since only the potential activity is measured and microbial processes are the result of multiple enzymatic reactions (Nannipieri et al. 2012). Furthermore, due to functional redundancy (Louca et al. 2018) it is possible, that the microbial activity still remained stable, despite the changes observed in bacterial community structure on family level. Whether also rare species play a role for functional redundancy is an interesting aspect for further studies and needs to be investigated in detail.

Changes in average maximum potential activity of β-glucosidase, acid phosphatase and β-N-acetylhexosaminidase with sampling time can be linked to plant growth stages and rhizodeposition. Plants secrete root exudates and thereby influence the microbial community and activity in the rhizosphere (Nannipieri et al. 2008). Furthermore, Philippot et al. (2013) showed that rhizodeposition changes throughout the plant life cycle and alongside with changes in microbial activity. Although the activity of β-glucosidase and acid phosphatase was weakest at the final sampling point (Fig. 3) an association to the bacterial families *Pyrinomonadaceae*, *Burkholderiaceae* and *Nitrosomonadaceae* (for MUP only) was found (Fig. 8). The decrease of β-glucosidase in line with a decrease of *Burkholderiaceae* might be explained by findings of Kim et al. (2006), who described members of this family as β-glucosidase producer.

Changes in bacterial abundance as response to plant growth

Within this study, the most pronounced effect on soil-rhizosphere microbiota was observed via molecular barcoding. While α-diversity (Shannon) of the soil bacterial community remained constant, different indices including species evenness, β-diversity, as well as comparative abundance analysis revealed strong changes. These were strongest between the initial and the final sampling (across all treatments). Since experimental conditions were controlled, and the initial soil represents only bulk soil while the final samples are a soil-rhizosphere mixture, the most pronounced difference seems to be plant development. It may therefore be hypothesized that the major changes in bacterial abundance are caused by plant growth and rhizodeposition, reflecting also the dynamics seen for the average maximum potential EEAs. Similarly, Philippot et al. (2013) reported strong influence of plants on rhizosphere microbiota in natural and agricultural ecosystems. However, also minor effects on family level arose among treatments including controls, which can be related to OA and MF application. Soil pH as important driver of bacterial community structure as shown by Lauber et al. (2009) is not as pronounced in this study because its values remained almost unchanged and no relationship could be detected with the most abundant families (Fig. 8).

Phylogenetic lineage analyses revealed highest abundances of phyla to be common in the soil environment similar to observations made by Fierer (2017), Lauber et al. (2009) and Obermeier et al. (2020). In addition, Buée et al. (2009) have shown that *Proteobacteria*, *Actinobacteria* and *Acidobacteria* are highly abundant in the rhizosphere with strong variations between treatments and studies. However, our study did not reveal any significant change for the phyla between treatments except when initial bulk soil was compared with the final soil-rhizosphere mixtures. This was most pronounced for the strong increase of the highly abundant phyla *Acidobacteria* in line with the decrease of *Actinobacteria*, *Chloroflexi*, and *Bacteroidetes* and most likely caused by plant growth. A strong decrease of *Chloroflexi* in the rhizosphere of different barley varieties compared to bulk soil has already been proven (Bulgarelli et al. 2015). Furthermore, the strong increase of *Acidobacteria* observed in the final soil mixtures can be explained by the input of inorganic and organic nutrients since its abundance is correlated to organic carbon availability (Kielak et al. 2016). The high root density in final soils is likely to promote this high carbon content by rhizodeposition (Philippot et al. 2013). In addition, Buée et al. (2009) showed that *Acidobacteria* are highly dominant in rhizospheres of different plant species which also may explain the observed increase. On class level the strong increase of *Alphaproteobacteria* observed in the final soil samples can also be related to carbon availability (Zhou et al. 2016). However, Bulgarelli et al. (2015) and Yang et al. (2017) showed that depending on the barley variety rhizosphere microbiota may strongly differ.

Similar to the observations made for the different phyla, the dynamics of bacterial families exhibited most pronounced changes when comparing the initial with the final sampling. A strong increase was observed for families *Xanthobacteraceae*, *Mycobacteriaceae*, and *Pyrinomonadaceae*. On the contrary, *Nitrosomonadaceae, Chitinophagaceae, Xanthomonadaceae,* and *Burkholderiaceae* were subject to the most pronounced decrease. Decreasing abundances of the ammonia-oxidizing family *Nitrosomonadaceae* during cultivation of some cover crops and after the application of organic fertilizer have been reported previously (Fernandez et al. 2016). In accordance with our findings, these authors also described the influence of the rhizosphere as being more pronounced than treatment with organic amendments on shaping the bacterial composition. Although the effects of the different treatments on bacterial community structure at the final sampling time were not as pronounced, interesting effects were observed for treatment B_MF140. Already species evenness (Fig. 4) and pH were lowest for this treatment, which exhibited highest relative abundances of the families *Xanthobacteraceae* and *Mycobacteriaceae* together with lowest abundance of *Haliangiaceae*. An association of the latter two families to nitrate-N has been shown (Fig. 8) and can be explained with the highest nitrate-N ratio remaining at the end of the experiment due to the highest amounts of mineral fertilizer application together with high amounts of biochar. A high relative abundance of *Mycobacteriaceae* (specifically *Mycobacterium*) in biochar amended soils has also been found by Anderson et al. 2011, who highlight the role of several *Mycobacterium* species as nitrate reducers. This indicates that following the development of the crop an application of mineral fertilizer together with higher amounts of biochar seem to strongly shape the bacterial community structure in this short-term experiment.

In conclusion, working hypothesis (1) could be confirmed showing maintained soil quality and improved plant performance after the application of organic amendments. Furthermore, hypothesis (2) needs to be differentiated since differences in the input of organic amendments alone or in combination with mineral fertilizer on microbial activity did not follow a clear pattern. However, the influence of higher doses of mineral fertilizer and biochar on shaping bacterial community structure could be proven (B_MF140). Hypothesis (3) needs to be revised since the potential extracellular enzyme activities did not depend on treatment (OA vs. MF) but were shown to be triggered by plant growth. Finally, hypothesis (4) must be denied since C/N ratios of the treatments did not influence microbial N immobilization.

Conclusion and outlook

The comprehensive approach of the present greenhouse study revealed strong changes of soil-rhizosphere microbiota dependent on plant growth and organic amendment application. Interestingly, plant performance was improved by all treatments but no difference between organic amendment application alone or with mineral fertilizer was observed. The majority of soil parameters remained stable throughout the study and across different fertilizer applications, indicating maintained soil quality. However, the strong shift of the bacterial composition between initial and final soils can be linked to plant growth and emphasizes the importance of considering plant species and taking its specific belowground parameters (e.g. root exudates) into account when analyzing or predicting effects of organic amendments on the soil-rhizosphere microbiota. Differences among treatments on family level were less pronounced and most likely triggered by the higher amounts of mineral fertilizer application in combination with biochar. The unaffected microbial activity in line with the changes seen for the bacterial families indicate microbial functional redundancy which is likely to promote and maintain soil quality. It further highlights the advantage of molecular barcoding approaches for elucidating changes in the soil environment.

The present study provides valuable insights in the response of the soil-rhizosphere microbiota upon fertilization and fosters our understanding of the complexity of plant-soil-microbe interaction. Long-term experiments have to scrutinize these findings under field conditions to find optimal fertilizer combinations for different agroecosystems.

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Author contributions MMO and ELM designed and performed experiments, analyzed the data and equally contributed in writing the manuscript. ADR helped analyzing the data. FR provided the laboratory for and instructed to fluorescein diacetate and BIOLOG analyses. PS provided funding, contributed to the design of the experiments, advised the studies and critically revised the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Appendix M2 (Supplements II)

Figures

Figure S1 Management and sampling scheme of the short-term greenhouse experiment in weeks (W1 – W8). Black arrows indicate seeding of 4 seeds (S4), picking of 2 seedlings (P2) and application of mineral fertilizer (MF). Red arrows mark time points for soil sampling including initial and final (together with plant harvesting) and non-destructive samplings for extracellular enzyme activity analysis (d0, d14, d28 and d42). Green arrows mark time points for chlorophyll measurements.

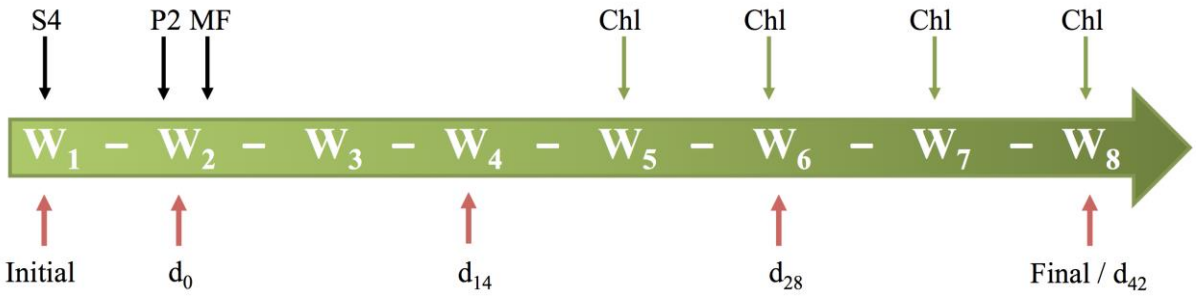


Figure S2 Correlation analysis of dry total plant biomass (TPB in g dw) with (A) leaf area index (LAI in % of green pixel), (B) nitrate-N (in µg g-1 dw) and (C) amount of biochar applied to the soil (in g kg-1) as well as the correlation of (D) nitrate-N to biochar (n=56). The 95% confidence interval, correlation coefficient (r) and its p-value are given.

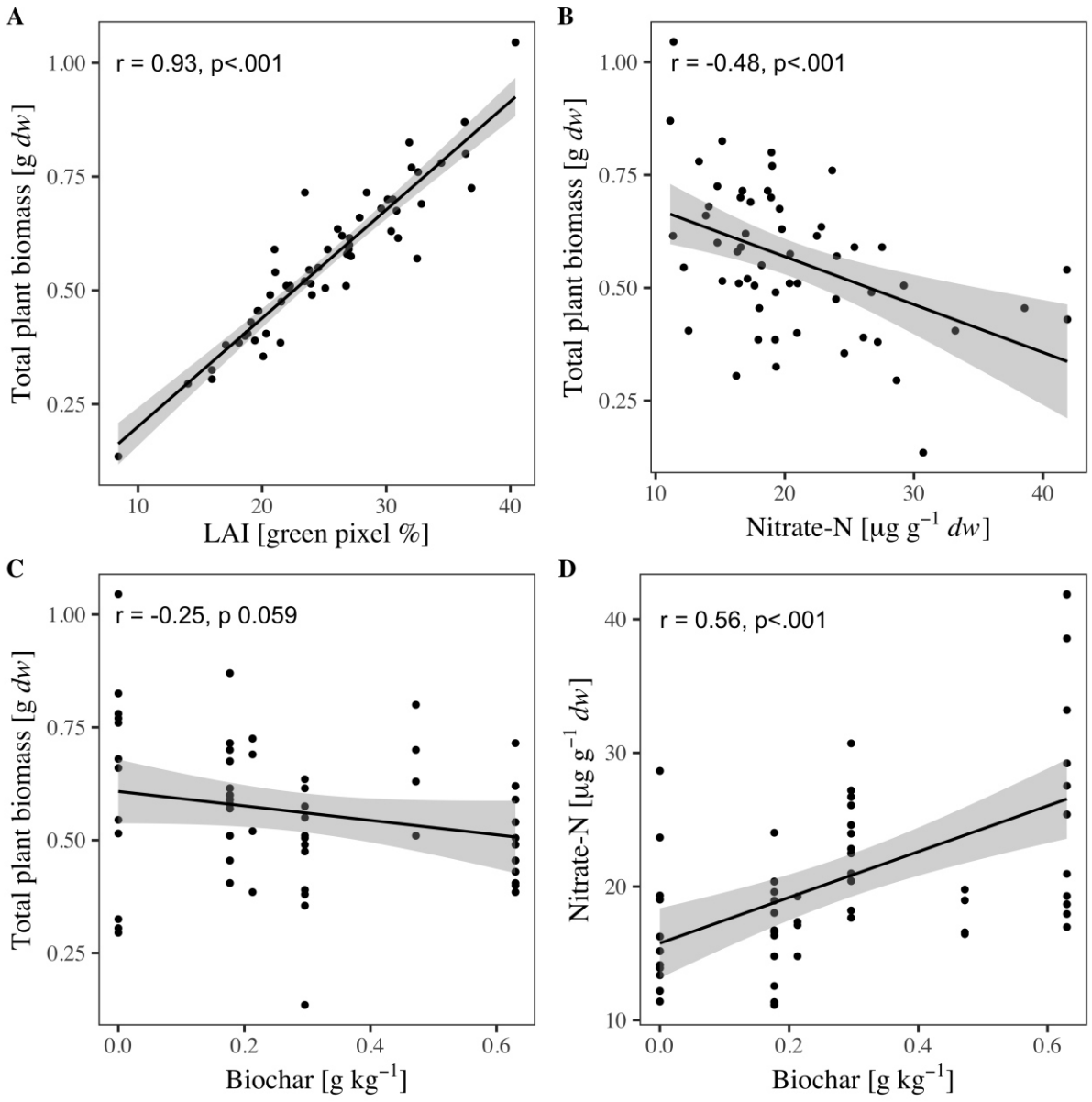


Figure S3 Ratio between treatments and mean of the initial soil for total dissolved nitrogen (TDN) with n = 4. Black dashed line indicates no difference to the initial soil. Different letters (a, b, c, d, and e) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

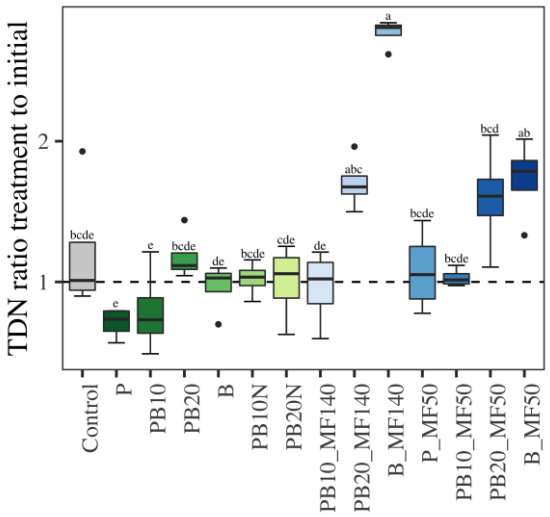


Figure S4 Potential soil enzyme activities. **(A)** Overall potential microbial activity in mg fluorescein kg^{-1} dw h^{-1} measured by a fluorescein diacetate (FDA) hydrolysis assay according to Green et al. 2006. Measurements were conducted in triplicates in 96-well plates at a wavelength of 490 nm using a FLUOstar™ Omega Plate Reader (BMG Labtech, Ortenberg, Germany). Potential activity was determined using a standard curve measured at 490 nm with 2, 5, 8, 11 and 15 µg mL^{-1} fluorescein. **(B)** Average well color development (AWCD) of carbon source metabolization using BIOLOG EcoPlates™ (Biolog Inc., Hayward, United States). Calculations were done according to Li et al. (2018) and Guckert et al. (1996). Different letters (a, and b) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

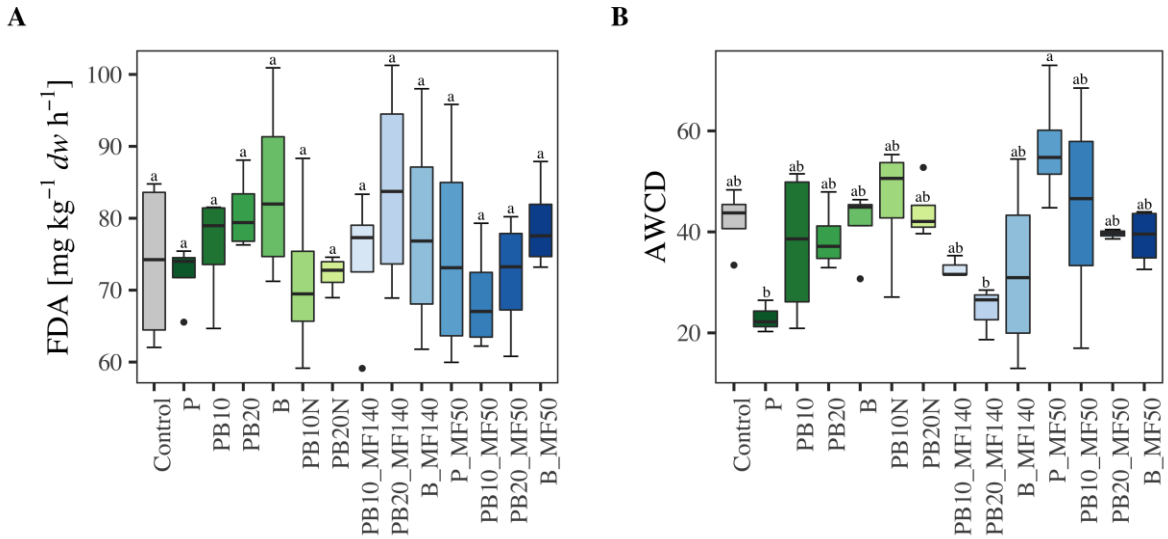


Figure S5 Rarefaction curves before subsampling to 45,750 sequencing reads per sample (see red dashed line).

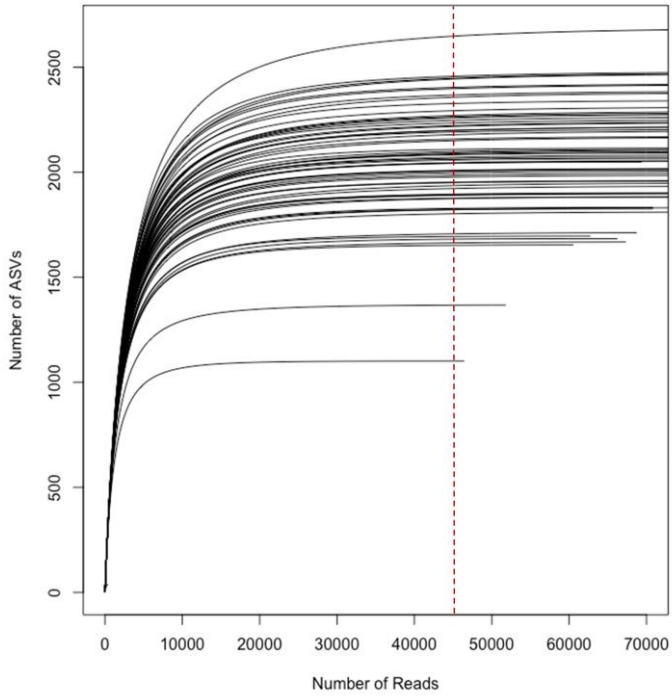


Figure S6 Bacterial α-diversity **(A)** Shannon diversity index and **(B)** observed ASVs for the 13 different fertilizer combinations, control and the initial soil (n = 4). No significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test) have been observed.

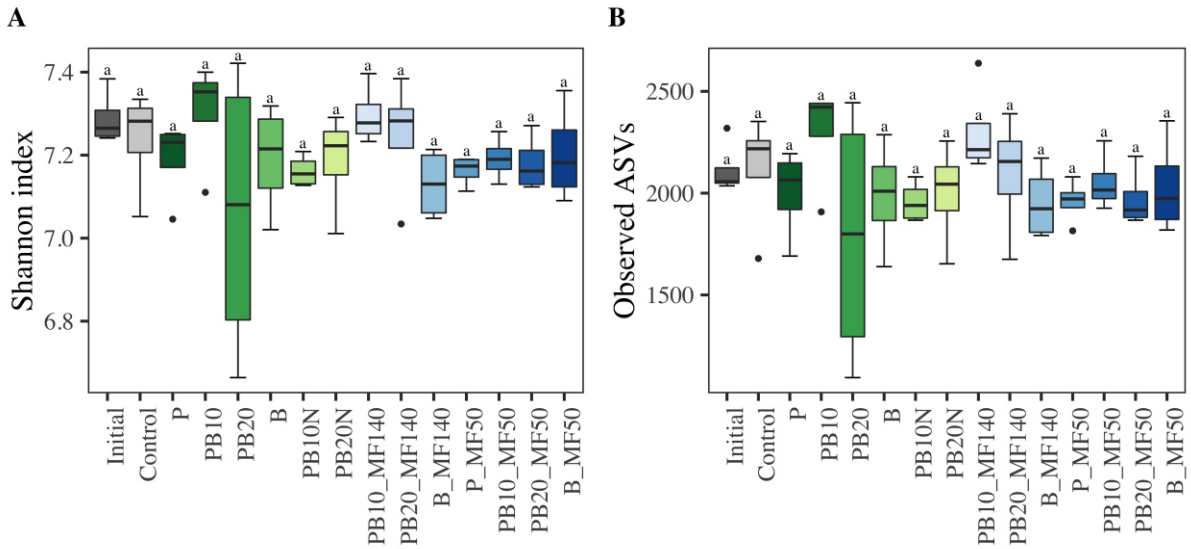
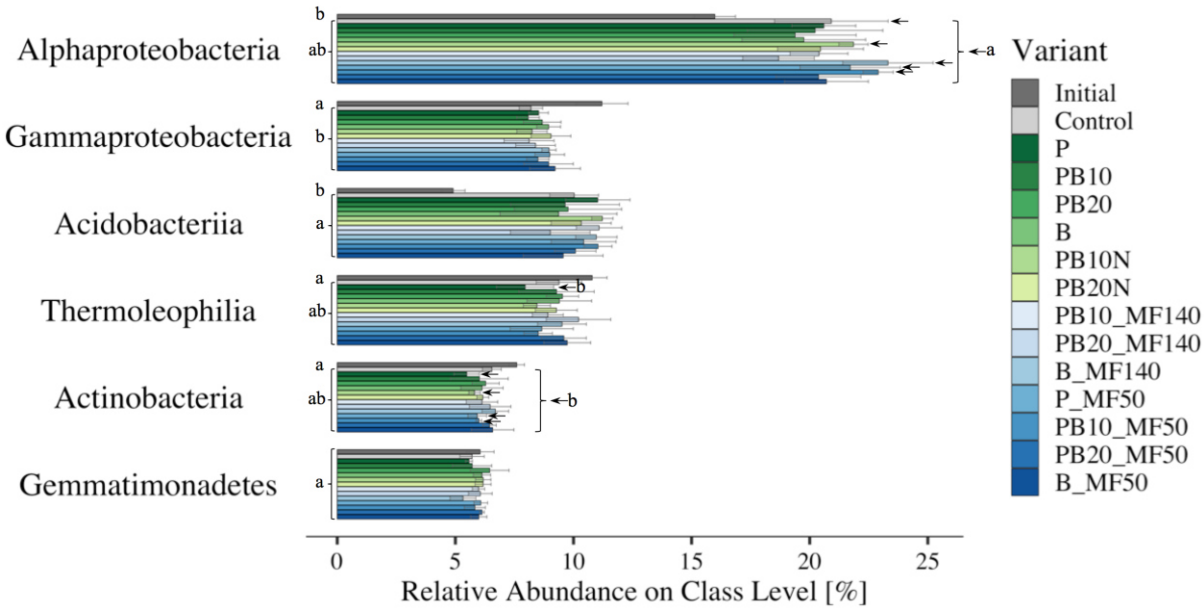


Figure S7 Mean relative abundances with standard deviations for the 6 most abundant classes observed for the 15 variants in percent (n = 4). Different letters (a and b) indicate significant differences ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test).



Tables

Table S1 Fertilization scheme of the short-term greenhouse experiment. Thirteen different fertilizer compositions of organic amendments (OA) only and in combination with mineral fertilizer (OA+MF). Organic amendments comprise biochar (B) and pellets (P) from 50% spent mushroom substrate, 30% bio-rest from biogas production and 20% straw and in part blended with biochar 10% (PB10) and 20% (PB20). Amount of used OA and MF in g kg^{-1} as well as total nitrogen (N_{tot}) and total carbon (C_{tot}) content in kg ha^{-1} for 1.5 t m^{-3} bulk density and 30 cm soil depth.

	Treatment	**OA [g kg^{-1}]**	**MF [g kg^{-1}]**	**N_{tot} [kg ha^{-1}]**	**C_{tot} [kg ha^{-1}]**
OA	P	2.10	-	140	2013
	PB10	1.77	-	116	2013
	PB20	1.48	-	88	2013
	B	0.63	-	6	2013
	PB10N	2.13	-	140	2424
	PB20N	2.36	-	140	3204
OA + MF	PB10_MF140	1.77	0.02	140	2013
	PB20_MF140	1.48	0.04	140	2013
	B_MF140	0.63	0.11	140	2013
	P_MF50	2.10	0.04	190	2013
	PB10_MF50	1.77	0.04	166	2013
	PB20_MF50	1.48	0.04	138	2013
	B_MF50	0.63	0.04	56	2013

Table S2 Determination of key quality parameters of soil and plant material. Given are min, max, mean and SD for soil measurements in µg g^{-1} *dw* and plant measurements with total plant biomass (TPB), shoot and root in g *dw*, shoot and root carbon and nitrogen in µg g^{-1} *dw*, LAI in green pixel % and chlorophyll (Chl) in µg cm^{-2} leaf area for sampling week 5, 6, 7 and 8. Mean values refer to average values with $n = 60$ and $n = 56$ for soil and plant measurements, respectively. Results of one-way ANOVA with degrees of freedom [*df*], F-statistic [*F*] and its p-value [*p*] using variant and treatment as factor for soil and plant measurements, respectively.

Measurement	**Min**	**Max**	**Mean**	**SD**	***df***	***F***	***p***
pH_{CaCl2}	4.86	5.45	5.13	0.13	14	2.03	0.037
Nitrate-N	11.13	41.88	21.39	7.29	14	12.71	<0.001
Ammonium-N	0.06	0.35	0.16	0.06	14	1.45	0.173
TDN	17.98	104.39	45.89	20.10	14	10.03	<0.001
DOC	4.35	54.13	24.93	8.65	14	0.83	0.638
DON	21.86	92.86	42.58	14.95	14	2.34	0.003
DOC:DON	0.17	1.33	0.62	0.25	14	0.77	0.693
MBC	405.67	809.72	587.57	85.59	14	1.14	0.355
MBN	17.83	155.36	62.09	20.54	14	0.91	0.554
MBC:MBN	3.37	28.06	10.30	3.41	14	0.73	0.747
TPB	0.14	1.04	0.56	0.16	13	3.60	<0.001
Shoot	0.12	0.92	0.51	0.15	13	3.80	<0.001
Root	0.01	0.13	0.05	0.02	13	1.43	0.135
C_{Shoot}	43.57	72.45	57.08	4.65	13	1.05	0.401
N_{Shoot}	7.63	11.88	9.55	0.65	13	0.65	0.810
$C:N_{Shoot}$	5.55	6.43	5.97	0.19	13	1.50	0.108
C_{Root}	65.24	142.31	90.90	16.12	13	1.13	0.324
N_{Root}	2.81	6.66	4.18	0.76	13	1.39	0.204
$C:N_{Root}$	19.92	24.03	21.79	0.76	13	1.52	0.150
LAI	8.41	40.41	25.18	6.33	13	3.28	0.002
Chl_{W5}	22.25	32.56	27.98	1.76	13	1.69	0.099
Chl_{W6}	24.41	36.25	31.78	2.17	13	1.65	0.109
Chl_{W7}	25.20	36.33	30.97	2.13	13	1.15	0.346
Chl_{W8}	26.06	34.95	30.06	2.26	13	1.21	0.310

Table S3 Measurements of soil enzyme activity. Given are min, max, mean and SD for FDA in mg fluorescein kg^{-1} h^{-1}, AWCD with Shannon and Evenness as well as β-glucosidase (MUG), β-N-acetylhexosaminidase (MUN) and acid phosphatase (MUP) in pmol MU g^{-1} h^{-1} for the different sampling times in days (d0, d14, d28, d42). Mean values refer to average values with n = 56. Results of one-way ANOVA with degrees of freedom [*df*], F-statistic [*F*] and its p-value [*p*] using treatment as factor.

Measurement	**Min**	**Max**	**Mean**	**SD**	***df***	***F***	***p***
FDA	59.12	101.27	75.94	10.38	13	0.79	0.669
AWCD	12.97	72.99	39.46	12.48	13	2.16	0.032
$Shannon_{AWCD}$	2.13	3.00	2.64	0.19	13	1.15	0.349
$Evenness_{AWCD}$	0.62	0.87	0.77	0.05	13	1.15	0.349
MUG_{d0}	55.69	144.98	103.14	19.08	13	0.84	0.618
MUG_{d14}	62.93	252.65	126.94	41.51	13	0.92	0.546
MUG_{d28}	49.87	224.90	103.58	35.58	13	1.31	0.197
MUG_{d42}	43.02	161.71	88.29	31.10	13	0.62	0.824
MUN_{d0}	0.53	8.59	4.00	1.36	13	1.24	0.245
MUN_{d14}	1.13	16.77	5.26	2.42	13	1.01	0.441
MUN_{d28}	1.24	8.57	3.59	1.53	13	1.28	0.214
MUN_{d42}	1.07	7.51	3.49	1.57	13	0.72	0.743
MUP_{d0}	18.39	87.13	57.80	14.75	13	1.11	0.381
MUP_{d14}	33.51	123.06	74.71	24.31	13	3.69	0.002
MUP_{d28}	21.70	149.62	60.70	25.97	13	1.12	0.338
MUP_{d42}	12.59	90.48	40.84	17.38	13	0.93	0.516

Table S4 Mean relative abundances [%] and standard deviations for the 12 most abundant families observed for the 15 variants (n = 4). Different letters (a, b, and c) indicate significant differences between all variants ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

	Xanthobacteraceae	*Gemmatimonadaceae*	*Nitrosomonadaceae*	*Solirubrobacterales (67-14)*	*Gaiellaceae*	*Mycobacteriaceae*	*Chitinophagaceae*	*Haliangiaceae*	*Nitrospiraceae*	*Pyrinomonadaceae*	*Xanthomonadaceae*	*Burkholderiaceae*
Initial	8.1 ± 0.4 ^{c}	6 ± 0.6 ^{a}	3.1 ± 0.2 ^{a}	2.8 ± 0.1 ^{a}	2.4 ± 0.2 ^{a}	1.4 ± 0.1 ^{c}	2.6 ± 0.3 ^{a}	1.3 ± 0.1 ^{bc}	2 ± 0.3 ^{a}	0.8 ± 0.1 ^{b}	1.8 ± 0.5 ^{a}	2.3 ± 0.3 ^{a}
Control	11 ± 1.2 ^{ab}	5.7 ± 0.5 ^{a}	2.4 ± 0.3 ^{ab}	2.4 ± 0.2 ^{ab}	2.2 ± 0.2 ^{a}	2.2 ± 0.3 ^{ab}	1.9 ± 0.1 ^{b}	1.6 ± 0.2 ^{ab}	1.4 ± 0.3 ^{ab}	1.4 ± 0.1 ^{ab}	0.8 ± 0.1 ^{c}	1 ± 0.2 ^{b}
P	10.7 ± 0.8 ^{abc}	5.6 ± 0.1 ^{a}	2.2 ± 0.3 ^{ab}	2 ± 0.4 ^{b}	2.1 ± 0.2 ^{a}	2.1 ± 0.1 ^{ab}	1.8 ± 0.2 ^{b}	1.7 ± 0.1 ^{ab}	1.6 ± 0.2 ^{ab}	1.8 ± 0.2 ^{a}	1 ± 0.2 ^{bc}	0.9 ± 0.1 ^{b}
PB10	10.7 ± 1.8 ^{abc}	5.7 ± 0.8 ^{a}	2.2 ± 0.6 ^{ab}	2.4 ± 0.3 ^{ab}	2.2 ± 0.3 ^{a}	2 ± 0.4 ^{bc}	1.8 ± 0.1 ^{b}	1.8 ± 0.2 ^{a}	1.4 ± 0.4 ^{ab}	1.5 ± 0.5 ^{a}	0.8 ± 0.1 ^{c}	1 ± 0.2 ^{b}
PB20	10.3 ± 1.9 ^{abc}	6.5 ± 0.8 ^{a}	2.5 ± 0.6 ^{ab}	2.5 ± 0.1 ^{ab}	2.3 ± 0.3 ^{a}	2.1 ± 0.4 ^{ab}	1.8 ± 0.2 ^{b}	1.6 ± 0.3 ^{ab}	1.5 ± 0.2 ^{ab}	1.6 ± 0.6 ^{a}	1.2 ± 0.3 ^{bc}	1.1 ± 0.3 ^{b}
B	10.4 ± 1.4 ^{abc}	6.1 ± 0.4 ^{a}	2.8 ± 0.6 ^{ab}	2.4 ± 0.4 ^{ab}	2.2 ± 0.3 ^{a}	2 ± 0.3 ^{abc}	1.8 ± 0.1 ^{b}	1.6 ± 0.1 ^{ab}	1.5 ± 0.3 ^{ab}	1.4 ± 0.3 ^{ab}	1.1 ± 0.3 ^{bc}	1 ± 0.2 ^{b}
PB10N	11.6 ± 0.2 ^{ab}	6.2 ± 0.3 ^{a}	2.1 ± 0.3 ^{b}	2.2 ± 0.2 ^{ab}	2.1 ± 0.2 ^{a}	2.2 ± 0.1 ^{ab}	1.6 ± 0.1 ^{b}	1.6 ± 0.2 ^{ab}	1.3 ± 0.1 ^{b}	1.5 ± 0.1 ^{a}	1 ± 0 ^{bc}	0.9 ± 0.1 ^{b}
PB20N	10.8 ± 1 ^{abc}	6.2 ± 0.3 ^{a}	2.4 ± 0.4 ^{ab}	2.4 ± 0.2 ^{ab}	2.1 ± 0.2 ^{a}	2.1 ± 0.3 ^{ab}	1.8 ± 0.2 ^{b}	1.6 ± 0.1 ^{ab}	1.4 ± 0.2 ^{ab}	1.5 ± 0.2 ^{a}	1.4 ± 0.1 ^{abc}	1 ± 0.2 ^{b}
PB10_MF140	10.9 ± 0.8 ^{ab}	6 ± 0.3 ^{a}	2.1 ± 0.3 ^{b}	2.3 ± 0.2 ^{ab}	2.2 ± 0.1 ^{a}	2.2 ± 0.3 ^{ab}	1.9 ± 0.3 ^{b}	1.6 ± 0.3 ^{ab}	1.3 ± 0.3 ^{b}	1.6 ± 0 ^{a}	1 ± 0.2 ^{bc}	0.9 ± 0.1 ^{b}
PB20_MF140	9.6 ± 0.9 ^{bc}	6.1 ± 0.5 ^{a}	2.5 ± 0.2 ^{ab}	2.7 ± 0.4 ^{ab}	2.3 ± 0.3 ^{a}	1.9 ± 0.2 ^{bc}	2 ± 0.2 ^{b}	1.3 ± 0.1 ^{bc}	1.7 ± 0.2 ^{ab}	1.3 ± 0.4 ^{ab}	1 ± 0.3 ^{bc}	1.1 ± 0.2 ^{b}
B_MF140	12.4 ± 1 ^{a}	5.3 ± 0.5 ^{a}	2.1 ± 0.4 ^{b}	2.3 ± 0.3 ^{ab}	2.3 ± 0.2 ^{a}	2.6 ± 0.3 ^{a}	1.8 ± 0.2 ^{b}	1 ± 0.1 ^{c}	1.2 ± 0.1 ^{b}	1.6 ± 0.4 ^{a}	1.5 ± 0.2 ^{ab}	1 ± 0.1 ^{b}
P_MF50	11.3 ± 1 ^{ab}	6.1 ± 0.3 ^{a}	2.3 ± 0.4 ^{ab}	2.2 ± 0.5 ^{ab}	2.1 ± 0.2 ^{a}	2.2 ± 0.2 ^{ab}	1.8 ± 0.2 ^{b}	1.7 ± 0.2 ^{ab}	1.4 ± 0.3 ^{ab}	1.5 ± 0.1 ^{a}	1.2 ± 0.2 ^{abc}	1 ± 0.1 ^{b}
PB10_MF50	12 ± 0.4 ^{ab}	5.8 ± 0.4 ^{a}	2.2 ± 0.1 ^{ab}	2.3 ± 0.2 ^{ab}	2.1 ± 0.2 ^{a}	2.4 ± 0.2 ^{ab}	1.8 ± 0.2 ^{b}	1.5 ± 0.1 ^{ab}	1.2 ± 0.2 ^{b}	1.6 ± 0.2 ^{a}	1.2 ± 0.2 ^{bc}	0.9 ± 0.1 ^{b}
PB20_MF50	10.6 ± 0.9 ^{abc}	6.1 ± 0.1 ^{a}	2.5 ± 0.2 ^{ab}	2.3 ± 0.2 ^{ab}	2.4 ± 0.2 ^{a}	2.2 ± 0.2 ^{ab}	2 ± 0.3 ^{b}	1.4 ± 0.1 ^{abc}	1.6 ± 0.2 ^{ab}	1.4 ± 0.1 ^{ab}	1.2 ± 0.3 ^{abc}	1 ± 0.1 ^{b}
B_MF50	10.7 ± 1 ^{abc}	6 ± 0.3 ^{a}	2.6 ± 0.2 ^{ab}	2.4 ± 0.3 ^{ab}	2.3 ± 0.2 ^{a}	2.2 ± 0.3 ^{ab}	2.1 ± 0.2 ^{b}	1.3 ± 0.1 ^{bc}	1.6 ± 0.1 ^{ab}	1.5 ± 0.2 ^{ab}	1.2 ± 0.3 ^{abc}	1.1 ± 0.1 ^{b}

Table S5 Ratio of the 12 most abundant families compared to the mean of the initial soil for 14 different treatments (n = 4) and the average trend across all treatments (final compared to initial). Different letters (a, b, and c) indicate significant differences between treatments ($p < 0.05$) calculated with multivariate ANOVA (Tukey's post-hoc test).

	Xanthobacteraceae	*Gemmatimonadaceae*	*Nitrosomonadaceae*	*Solirubrobacterales (67-14)*	*Gaiellaceae*	*Mycobacteriaceae*	*Chitinophagaceae*	*Haliangiaceae*	*Nitrospiraceae*	*Pyrinomonadaceae*	*Xanthomonadaceae*	*Burkholderiaceae*
Control	39.5 ± 14.8 ^{ab}	-7.4 ± 8.3 ^{a}	-20.9 ± 11 ^{a}	-14.3 ± 6 ^{a}	-7.9 ± 7.8 ^{a}	58.7 ± 22.7 ^{ab}	-29.5 ± 5 ^{a}	25.3 ± 18.8 ^{ab}	-28 ± 16.1 ^{a}	85.9 ± 17.5 ^{a}	-54.3 ± 4.8 ^{c}	-58.5 ± 6.7 ^{a}
P	35.1 ± 10.2 ^{ab}	-9.5 ± 2.4 ^{a}	-29.5 ± 10.5 ^{a}	-29.9 ± 14.1 ^{a}	-13.4 ± 8.5 ^{a}	55.6 ± 9.5 ^{ab}	-32.7 ± 7.7 ^{a}	28.9 ± 9 ^{ab}	-21.6 ± 11.9 ^{a}	131.4 ± 28.2 ^{a}	-42.2 ± 13.4 ^{abc}	-60.3 ± 4.6 ^{a}
PB10	34.6 ± 22.1 ^{ab}	-7.3 ± 13.4 ^{a}	-27.2 ± 19.3 ^{a}	-17.3 ± 12 ^{a}	-5.3 ± 13.1 ^{a}	42.9 ± 27.5 ^{b}	-33.3 ± 3.5 ^{a}	36 ± 12.6 ^{a}	-27.7 ± 18.5 ^{a}	102.2 ± 71 ^{a}	-52.8 ± 6 ^{bc}	-57.7 ± 8.2 ^{a}
PB20	30.5 ± 23.8 ^{ab}	4.7 ± 13.3 ^{a}	-19 ± 20.3 ^{a}	-11.3 ± 4.8 ^{a}	-1.1 ± 10.7 ^{a}	51.3 ± 25.8 ^{ab}	-33.3 ± 8.1 ^{a}	22.5 ± 23.2 ^{ab}	-24.2 ± 12.6 ^{a}	113.3 ± 76.9 ^{a}	-35.2 ± 15.3 ^{abc}	-53 ± 11.6 ^{a}
B	31 ± 18.3 ^{ab}	-0.6 ± 5.9 ^{a}	-9.1 ± 18.3 ^{a}	-16.8 ± 12.9 ^{a}	-5.9 ± 12.4 ^{a}	47.3 ± 20.5 ^{ab}	-31.6 ± 4.5 ^{a}	22.9 ± 7.3 ^{ab}	-22.1 ± 15.4 ^{a}	81.2 ± 36.9 ^{a}	-39.9 ± 14.8 ^{abc}	-56.2 ± 7.3 ^{a}
PB10N	46 ± 1.9 ^{ab}	0.2 ± 5.3 ^{a}	-31.4 ± 8.3 ^{a}	-23 ± 8.6 ^{a}	-9.7 ± 9.6 ^{a}	59 ± 9.8 ^{ab}	-38.3 ± 2.8 ^{a}	23.9 ± 16.7 ^{ab}	-33.8 ± 5.2 ^{a}	99.8 ± 9.6 ^{a}	-43.9 ± 2.7 ^{abc}	-61.5 ± 4.3 ^{a}
PB20N	36.1 ± 12.3 ^{ab}	0.1 ± 5.5 ^{a}	-23.2 ± 14.3 ^{a}	-16.4 ± 7.4 ^{a}	-9.7 ± 10.4 ^{a}	50.3 ± 19 ^{ab}	-33.5 ± 9.1 ^{a}	27.9 ± 10.9 ^{ab}	-27 ± 10 ^{a}	99.5 ± 24.3 ^{a}	-22.8 ± 4.5 ^{ab}	-57.7 ± 10.7 ^{a}
PB10_MF140	37.7 ± 10.4 ^{ab}	-3 ± 4.2 ^{a}	-32.1 ± 9.8 ^{a}	-18.9 ± 5.3 ^{a}	-5.8 ± 6.2 ^{a}	59.9 ± 21.4 ^{ab}	-28.7 ± 10.6 ^{a}	23.2 ± 20.5 ^{ab}	-32.4 ± 16 ^{a}	104.2 ± 2.6 ^{a}	-44.2 ± 11.5 ^{abc}	-59.3 ± 6.2 ^{a}
PB20_MF140	20.7 ± 11 ^{b}	-1.7 ± 8.1 ^{a}	-17.7 ± 5.8 ^{a}	-6.4 ± 13.6 ^{a}	-2 ± 13.7 ^{a}	35.8 ± 11.8 ^{b}	-26 ± 8.1 ^{a}	1.3 ± 9.1 ^{bc}	-13.4 ± 8.4 ^{a}	73.3 ± 45.8 ^{a}	-44.5 ± 17.6 ^{abc}	-54.7 ± 8 ^{a}
B_MF140	56.6 ± 13.2 ^{a}	-13.7 ± 8.9 ^{a}	-33.3 ± 11.5 ^{a}	-17.5 ± 12 ^{a}	-2.9 ± 8.8 ^{a}	91.7 ± 21.8 ^{a}	-32 ± 6.3 ^{a}	-18.7 ± 4.2 ^{c}	-40.6 ± 7.2 ^{a}	107.3 ± 55 ^{a}	-15.8 ± 13.8 ^{a}	-57.3 ± 4.3 ^{a}
P_MF50	43.2 ± 12.7 ^{ab}	-1.4 ± 4.7 ^{a}	-25.9 ± 11.8 ^{a}	-23 ± 17.1 ^{a}	-9.4 ± 8.7 ^{a}	62.9 ± 16.6 ^{ab}	-31.6 ± 8.6 ^{a}	28.5 ± 14.3 ^{ab}	-29 ± 17.5 ^{a}	95.4 ± 18.8 ^{a}	-32.6 ± 9.6 ^{abc}	-59.3 ± 6.3 ^{a}
PB10_MF50	50.9 ± 4.8 ^{ab}	-5.5 ± 7.2 ^{a}	-29.6 ± 2 ^{a}	-19.5 ± 6.7 ^{a}	-11.9 ± 9.6 ^{a}	73 ± 12.8 ^{ab}	-31.5 ± 7 ^{a}	18.2 ± 10.3 ^{ab}	-37.7 ± 11.7 ^{a}	112.7 ± 20.4 ^{a}	-35.8 ± 9.9 ^{abc}	-62 ± 4.1 ^{a}
PB20_MF50	34.2 ± 11.6 ^{ab}	-0.6 ± 1.6 ^{a}	-18 ± 6.9 ^{a}	-17.6 ± 5.8 ^{a}	-0.1 ± 7.2 ^{a}	57.2 ± 16.2 ^{ab}	-25.3 ± 10.1 ^{a}	4.9 ± 5.5 ^{abc}	-20.8 ± 9.3 ^{a}	78.7 ± 6.6 ^{a}	-32.6 ± 15.3 ^{abc}	-55.8 ± 4.3 ^{a}
B_MF50	35.2 ± 12.5a ^{ab}	-2.8 ± 5.6 ^{a}	-14.7 ± 5.1 ^{a}	-15.3 ± 10.4 ^{a}	-1.8 ± 7.4 ^{a}	61.2 ± 19.5 ^{ab}	-21 ± 9.1 ^{a}	0.4 ± 7.6 ^{bc}	-19.4 ± 7.5 ^{a}	93.2 ± 28.2 ^{a}	-33 ± 18 ^{abc}	-54.2 ± 3.3 ^{a}
Average	**38.0 ± 15.1**	**-3.5 ± 8**	**-23.7 ± 12.9**	**-17.7 ± 10.6**	**-6.2 ± 9.5**	**57.6 ± 21.1**	**-30.6 ± 7.8**	**17.5 ± 18.7**	**-27.0 ± 13.2**	**98.4 ± 37**	**-37.8 ± 14.8**	**-57.7 ± 6.6**

Table S6 Differences among treatments observed with edgeR and given by log2FC, logCPM, p-value and false discovery rate (FDR). Shown are bacterial families with a FDR-value lower 0.05. Families belonging to the 12 most abundant families are written in bold. Asterisks (*) indicate seven families that revealed most pronounced differences for treatment B_MF140 compared to the other treatments.

Family	log2FC	logCPM	p-value	FDR
*BIrii41 **	-2.85	11.27	<0.0001	<0.0001
*Sandaracinaceae **	-0.82	11.58	0.0002	0.0110
*Reyranellaceae **	0.56	12.81	0.0017	0.0417
***Mycobacteriaceae **	0.42	14.55	0.0019	0.0417
bacteriap25	0.34	13.42	0.0020	0.0417
*Bdellovibrionaceae **	-1.03	10.34	0.0022	0.0417
NS11_12_marine_group	1.80	8.26	0.0034	0.0493
***Haliangiaceae **	-0.38	13.78	0.0035	0.0493
*Anaerolineaceae **	-1.92	9.41	0.0042	0.0493
Nitrosomonadaceae	-0.44	14.55	0.0044	0.0493

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iii. Appendix M3 (Manuscript III)

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Opinion Paper

Intensify production, transform biomass to energy and novel goods and protect soils in Europe—A vision how to mobilize marginal lands

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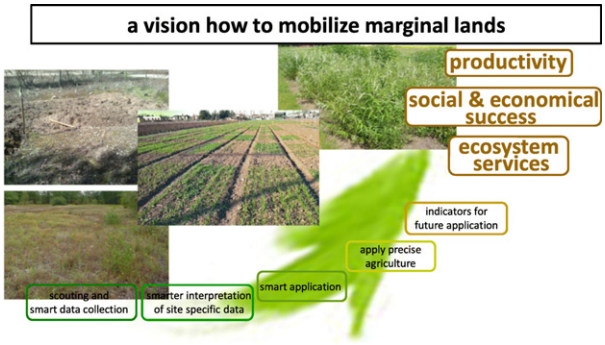
HIGHLIGHTS

- Challenges for smart intensification of marginal land are manifold
- Tools for precise agriculture will aid to detect pollutant hotspots and poor soils
- Crop rotation and adapted crop choice will yield biomass
- Amendments will sequester carbon and release fertilizer when needed
- Potentials of marginal soils can be unlocked and lead to ecological and economical success

GRAPHICAL ABSTRACT



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ABSTRACT

The rapid increase of the world population constantly demands more food production from agricultural soils. This causes conflicts, since at the same time strong interest arises on novel bio-based products from agriculture, and new perspectives for rural landscapes with their valuable ecosystem services. Agriculture is in transition to fulfill these demands. In many countries, conventional farming, influenced by post-war food requirements, has largely been transformed into integrated and sustainable farming. However, since it is estimated that agricultural production systems will have to produce food for a global population that might amount to 9.1 billion by 2050 and over 10 billion by the end of the century, we will require an even smarter use of the available land, including fallow and derelict sites. One of the biggest challenges is to reverse non-sustainable management and land degradation. Innovative technologies and principles have to be applied to characterize marginal lands, explore options for remediation and re-establish productivity. With view to the heterogeneity of agricultural lands, it is more than logical to apply specific crop management and production practices according to soil conditions. Cross-fertilizing with conservation agriculture, such a novel approach will provide (1) increased resource use efficiency by producing more with less (ensuring food security), (2) improved product quality, (3) ameliorated

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Surplus production
Soil amendments

nutritional status in food and feed products, (4) increased sustainability, (5) product traceability and (6) minimized negative environmental impacts notably on biodiversity and ecological functions. A sustainable strategy for future agriculture should concentrate on production of food and fodder, before utilizing bulk fractions for emerging bio-based products and convert residual stage products to compost, biochar and bioenergy. The present position paper discusses recent developments to indicate how to unlock the potentials of marginal land.

Contents

1. Introduction

"When soils fail, civilizations fall". This phrase, coined in 1937 by US president Franklin D. Roosevelt under the shock of the "American Dust Bowl" that had destroyed millions of hectares of arable land in the Midwest US is still of topical relevance today and a threatening reminder to protect our valuable production base for nutrition, drinking water supply and important ecosystem services.

All across the world, agriculture is in transition. Until now, conventional farming, influenced by the post-war food requirements, has largely been transformed into integrated and organic, sustainable farming, at least in the EU and advanced countries (Schröder et al., 2008a, 2008b). In 2011, 12 billion tons (t) dry matter (DM) biomass from agriculture, grazing and forestry have been utilized for feed (58%), bioenergy (heat and electricity, 16%), food (14%), material use (10%) and biofuels (1%) worldwide. The share of biofuels has reached 2%, and biomass used for industrial purposes in 2011 was 1.26 million t DM. But the rapidly increasing world population constantly demands even more food production from agricultural soils, sold to retailers at very low prices. This causes conflicts, since at the same time strong interest arises on novel bio-based products from farms, and new perspectives for the valuable ecosystem services of rural landscapes (De Marsily and Abarca-del-Rio, 2016) and soils (Mol and Keesstra, 2012).

Cascading, upgrading and recycling of bio-based products (SCAR-report, 2015) are visions for a novel circular economy, where the term "waste" has lost its former meaning. However, a sustainable strategy for future agriculture should always be to first use harvests for food and fodder, before utilizing biomass for emerging products (bioplastic, biochemicals, biomaterials, etc.). Next stage products are converted to compost, biochar and bioenergy. Roughly, the average value of 11.3 EJ of residues is estimated as available in Europe, equal to an energy content of about 269 MTOE (million tons oil equivalent). The current bioeconomy market is estimated at about € 2.4 billion, including agriculture, food and beverage, agroindustrial products, fisheries and aquaculture, forestry, and wood-based industry. In addition, biochemicals, enzymes, biopharmaceuticals, biofuels and bioenergy are produced, using about 2 billion tons of biomass and employing 22 million persons (Scarlat et al., 2015). The development trend of emerging bio-based

sectors foresees a total biomass demand for 2050 of about 290–320 MTOE. Finally, it is estimated that agricultural production systems will have to produce food for a global population that might amount to 9.1 billion people in 2050 and over 10 billion by the end of the century (UNFPA, 2011). A severe problem that cannot be tackled here is the fact that only 30% of the food produced reaches our stomachs – valuable agricultural goods are lost due to post-harvest problems, discarded due to presumed low quality, or rotten due to lacking distribution channels (SAVE FOOD, 2015). Increased agricultural production will require changes in our general attitude towards food products, smarter use of the available land, and a higher attention to avoid falling back in the mistakes of the past.

In future, land use has to embrace efficient production and utilization of biomass for improved economic, environmental and social outcomes. We will have to focus on integrated, systems-based approaches of land management with sustainable intensification of agricultural production, even on neglected sites: underexploited grassland, abandoned and set aside lands and brownfields with actual or aged pollution. Hence, marginal situations develop as the result of the interaction of a combination of factors (Brouwer et al., 2011). They all have in common that the land has lost its economical and/or ecological viability for the community, a situation that is complicated by the fact that such land is usually further degrading and ceases to contribute ecosystem services.

The potential of such sites has to be unlocked by innovative and sustainable production systems, open for a wide range of novel products and services. At the same time, relevant ecosystem services have to be conserved or strengthened. Merging natural with human made solutions will be needed to find a way to make our ecosystems compatible between nature and human use (Keesstra et al., 2018). Hence, challenges for smart intensification exist on many levels, and have to relate to the actual market developments. Farmers, policy makers, as well as all stakeholders including consumers have to contribute to novel solutions.

1.1. Challenges for smart intensification

Having postulated that the best soils should always be used for food production, while less productive fields could serve as production sites for biomass or energy, we have to understand why some lands are unproductive. One of the most severe impacts of expanded production and non-sustainable management is land degradation, which reverses the gains obtained from converting forest or grassland to agricultural use or in the passage from intensive to organic farming, and will threaten yield increases obtained from nutrient enrichment and better use of genetic resources (McLaughlin and Kinzelbach, 2015). Therefore, it is vital to support and improve cropland management without further degrading soil and depleting water resources. In the EU, the Joint Programming Initiative on Agriculture, Food Security and Climate Change (FACCE-JPI) aims to steer research to support sustainable agricultural production and economic growth, while maintaining and restoring ecosystem services under future climate change. Such an approach will promote sustainable agriculture with the potential to deliver ecosystem services in the form of reduced GHG emissions and increased carbon sequestration, contributing to climate change mitigation and adaptation (Branca et al., 2011; Campbell et al., 2014; Paustian et al., 2016).

Innovative technologies and principles aid to identify spatial and temporal variability in crop production. Once having recognized the heterogeneity of agricultural lands, it is more than logical to apply specific management practices at a given site according to soil conditions. Cross-fertilizing with conservation agriculture, such a novel approach will: increase resource use efficiency by producing more with less (ensuring food security), reaching targeted product quality, improve nutritional status in food and feed products, augment sustainability, raise product traceability and minimize negative environmental impacts notably on biodiversity and ecological functions.

Regarding climate change, one of the major challenges for agriculture is to diminish loss of carbon into the atmosphere after changes in soil tillage. Hence, there are numerous attempts to decrease the flux of carbon and nitrogen to the atmosphere from cropland, and, on the other hand, to sequester carbon in agricultural soils (Smith and Falloon, 2005). Among those options, management practices like reduced and zero tillage, setting-aside, perennial crops, deep rooting crops, addition of organic amendments (animal manure, sewage sludge, cereal straw, compost and biochar), improved rotations, irrigation, bioenergy crops, organic farming, are the most prominent (Smith and Falloon, 2005). The sequestration potential is up to 45 Tg (C) per year.

BOX 1 The nature of soils.

Soil is the biologically active, unconsolidated surface of the Earth. Well-developed mineral soil consists of 90% mineral and 10% bio-organic substance. The bio-organic part consists of 70–90% humus, 10–30% roots, and an active fraction, constituted of living soil organisms. However, in cool and humid regions, organic soils based on drained bogs can consist of close to 100% organic materials. Topsoil (0–30 cm) is the most important fraction, since it harbors the main turnover processes. Its basic quality depends on long term stability of humus, soil structure and organismic interactions. Soil fertility and productivity are both determined by a plethora of interconnected features including nutrient balance and release capability in the soil, soil acidity, organic matter content, soil structure, water retention, etc. (Havlin et al., 2013). The long-term functionality of all these soil processes in agricultural systems is highly dependent on healthy microbial activity (Van der Heijden et al., 2008). The soil and plant microbiome, i.e. all microorganisms present in soil, rhizosphere and plant, fulfill crucial roles in ecosystem functioning, nutrient cycling, plant nutrient uptake and disease suppression, which ultimately regulates plant health, physiology and performance (Berendsen et al., 2012; Bulgarelli et al., 2013; Raaijmakers et al., 2008; Bakker et al., 2013; Kiely et al., 2006). Soils promote and support vegetation, and strong relationships exist between habitats of high conservation value and soil properties. When soils are disturbed e.g. by pollutants, poor agricultural techniques or overexploitation, then due regard needs to be given to their restoration and recovery to ensure satisfactory re-establishment of habitats and future sustainable management (Puri, 2002). If this fails, the soil will become marginal, i.e. land will lose its viability with regards to economical or ecological demands of the farmer and the community. Four basic processes govern all ecosystems: mineral cycle, water cycle, energy flow and community dynamics, all of them have to be in harmony to guarantee the life on Earth. Especially the latter is under scrutiny today, but we are far from understanding which part of the soil diversity is key for soil functioning (Bender et al., 2016). For living beings to thrive, they need effective energy flow to feed them, a water cycle that supplies adequate moisture, and a mineral cycle that supplies vital nutrients. If this is not the case, the system will be imbalanced. If any of these processes is modified by negligence and poor ecosystem husbandry, it will automatically influence all of them, and the system will lose its resilience. Soil as a whole is a limited resource and its health is critical for any sustainable development, it is considered a no-renewable resource. To feed one person per year, 0.26 ha of fertile soil is needed (FAO, 1994).

In this context, a controversial discussion is ongoing whether grassland soils are richer in carbon than soils hosting any other crop types. While some authors find that forage crops store more carbon than any

Fig. 1. Typical examples for agricultural settlements on high yield lands. (A) Left: reconstruction of 6th–7th century Bajuvaric settlements in fertile plains close to Munich (www.bajuwarenhof.de, photo: PS). (B) Right: Historic BayernAtlas map of typical agricultural landscape close to Straubing, Bavaria, where those settlements were typically located in the middle of the fertile land, riverbanks, colluvial valleys and where still farm communities thrive (source: Geobasisdaten: Bayerische Vermessungsverwaltung). (C) Below left: Land use pattern in North western Spain – soil heterogeneity and topography lead to scattered land use and abandonment in case of drought stress (source: Instituto Geográfico Nacional – IGN, 2016). (D) Below right: Even under constricted topographical conditions (bedrock/sea) in western Norway, recent agricultural settlements consume fertile agricultural areas (photo: AS).

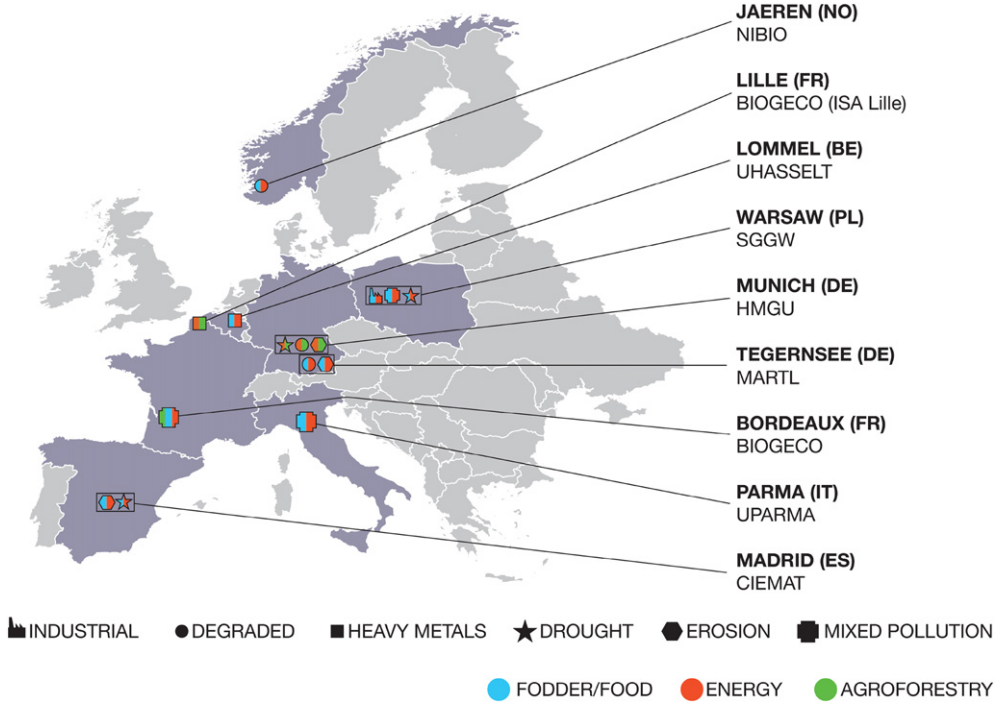


Fig. 2. Location of the study sites in the ERA-NET Cofund Project INTENSE with their main sustainability problems.

other crop except for grasslands (Gardi et al., 2016), others conclude that geographic distribution and climatic conditions may be more important. Soils in United Kingdom and Ireland (UKI) seem to contain significantly more carbon than soils e.g. in the Mediterranean region. Baltic and Scandinavian soils have more carbon than Atlantic Europe, Continental and Mediterranean Europe, but still less than UKI (Gardi et al., 2016). The potential to increase soil organic content (SOC) by land management practices seems to be generally higher in Central Europe compared to Southern or Northern Europe. While there is considerable potential in European croplands to sequester carbon in soils, it must be clear that carbon sequestration has a finite potential which is non-permanent. Furthermore, improved agricultural management often has a range of other environmental and economic benefits in addition to climate mitigation, and this makes any attempt to improve soil carbon storage attractive as part of integrated sustainability policies. Well-managed agricultural landscapes can also provide protection against extreme natural events like drought, storms and flooding. Clearly, trade-offs and synergies among ecosystem services need to be more fully understood and addressed hierarchically.

Covering major aspects of this complex issue, this position paper sketches soil problems, indicators of degradation and resilience, management strategies, soil amendments, and solutions for certain scenarios of European marginal lands.

2. Status of European soils: A plea for smarter biodiversity and soil management

2.1. Marginal lands

Marginal lands generally refer to areas not only with low production, but also with limitations that might make them unsuitable for agricultural practices and important ecosystem functions (Heimlich, 1989). Across Europe, marginalization caused severe losses of arable land as well as permanent meadows and pastures in the past. Overall, all forms of degradation amounted to about 10 million ha per year, which was not counterbalanced by the recovery of set aside land since 2008. Main causes of soil degradation have been identified to be: overgrazing (35%), agriculture (28%), deforestation (30%), producing fuel wood (7%), and industrialization (4%) (IP/B/AGRI/IC/2009_26, 2009). Similar results were reported by Longobardi et al. (2016). Based on estimates by the European Environment Agency (Bardos et al., 2008), the number of sites where potentially polluting activities have been carried out in the EU is approximately three million and, of these, an estimated 250,000 sites may need urgent remediation (Panagos et al., 2013). Costs for remediation projects of polluted sites usually range from €50,000 to €500,000 per site (40% of reported cases). Hence, the problem has been recognized, but not solved. In any case, degraded soil is less suited to prevent droughts and flooding and more prone to lose biodiversity (EEA, 2012).

It has been common practice, until 2007, to abandon sites of low productivity, and finally the area under obligatory set-aside amounted to 3.8 million hectares in the EU (Keenleyside et al., 2010). Considering average trends, yields from such areas would likely bring around 10 million t of grain onto the market (IP/07/1402, 2007). However, in many places the potential yields are not reached although improved practices could probably result in much larger productivity. Hence, marginal lands have recently been recognized for their potential to improve food security and support bioenergy production. Although a promising perspective, environmental issues, concern about losses of ecosystem services, and reduced sustainability have also been discussed in the context of using marginal land (Kang et al., 2013).

Given the large areas of degraded land, a huge opportunity in developing and implementing practices aimed at restoring the production potential exists. Such a restoration could be a major contribution to unlock increased production of food, bioenergy and other ecosystem services from land (Kidd et al., 2015). Hence, and following consequently the strategy of the FACCE agenda, a change in the EU's agricultural policies is needed to consider marginal, neglected or polluted sites for agricultural production, at least for raw materials and/or bioenergy, if not for feed and food.

2.2. Soil degradation by poor land husbandry

Ancient farmers settled close to their fields and meadows, in areas of highest soil fertility (Fig. 1). In Europe, this pattern remains largely unchanged, and recent settlements in rural areas still occupy a lot of good agricultural land. It has been long debated that the best soils are frequently sealed by different types of infrastructures, roads, industry, settlements, instead of utilizing them for sustainable production. Besides, in industrialized countries where agricultural foods are abundant and easy to reach for everybody, the production base seems to be neglected more and more. But poor land husbandry will have various effects on different soil types (Scherr, 1999), and possibilities of soil improvement can vary substantially, depending upon soil resilience (the resistance to degradation) and soil vulnerability (the degree to which soils degrade when subjected to degradation processes).

Degradation processes that can be aggravated by agricultural activity include water and wind erosion, physical and chemical weathering, and salt accumulation (Lal, 1989). Soil erosion is a land degradation process often found in cultivated environments due to natural processes (e.g. climate events) and accelerated by human activities (e.g. extensive tillage). It may reduce crop production potential, lower surface water quality and damage drainage systems (Toy et al., 2002). Extensive tillage over extended times may encompass loss in soil nutrients and organic matter which are stability factors, especially for the topsoil.

Topsoil is important for both, agricultural productivity and other soil functions, such as supporting amenity or nature conservation. Its damage will lead to irreparable long-term loss of an irreplaceable resource, since topsoil contains the majority of soil organic matter (carbon) (Jobbágy and Jackson, 2000) and most of the biological communities responsible for nutrient cycling and maintaining soil structure. Loss of organic matter, soil biodiversity and consequently soil fertility are often

Table 1
List of the study sites in the INTENSE project.

Name	Site	Climate	Lithology	Coordinates Lat/Long	Alt.(a.s.l)
Martl-Hof	DE1	Alpine	Calcareous	47°44′36″/11°45′41”	784
Roggenstein	DE2	Continental	Gravel	48°10′49″/11°19′07”	540
Buendía	ES1	Mediterranean	Limestone	40°22′10″/2°46′19”	732
Casasana	ES2	Mediterranean	Limest./gypsum	40°31′44″/2°38′11”	954
St.Médard d'Eyrans	FR1	Oceanic	Gravel	44°43′/0°30′	3–51
Parc aux Angéliques	FR2	Oceanic	Technosol	44° 51′ 20″/0° 33′ 7″	5
MetalEurop	FR3	Oceanic	Clays	50°26′15″/3°10′5.7”	28–40
Azienda Agraria Sperimentale Stuard	IT1	Continental	Alkaline silty-clay	44°48′28″/10°16′28”	60
Særheim	NO1	Oceanic	Glacial moraine	58°46′N/5° 39′E	90
Skiernivice	PL1	Continental	Stagnic Luvisol	51°95′N/20° 15′E	128

driven by unsustainable practices such as deep ploughing on fragile soils or cultivation of erosion-facilitating crops such as maize, and continuous use of heavy machinery destroys soil structure through compaction (German Advisory Council Global Change, 1994). Soil aggregation indices can be used as key-indicators for degradation processes in top soils at a fine scale with implications for runoff and sediment generating processes at hillslope scale. The degradation of soil aggregates is one of the primary processes in the loss of organic matter caused by long-term cultivation and overgrazing, but data on how formation and stabilization of macro-aggregates control C enrichment when disturbance is reduced are scarce. Inputs of organic matter, e.g. plant debris, might rapidly stimulate the formation of particles or colloids that are associated with minerals, are physically protected, slowed down in decomposition and promote the development of stable micro-aggregates. Although

Fig. 3. Spain. The test sites (ES1 and ES2) are in Central Spain in the autonomous region of Castilla La Mancha, under Mediterranean climate with a continental character. Site ES1 is located next to the town of Buendía (Fig. 3A) in the province of Cuenca 135 km northeast of Madrid. The relief is hilly and the site is gently sloping. The mean annual temperature and precipitation is 14 °C and 610 mm, respectively. The lithological substrate is mainly formed from the Inferior Miocene with red clays, gypsum clays and gypsum. Soils have a clay loam texture with a pH of 8.4 and an abundance of $CaCO_3$ of 30%. The site is within a mosaic of forests, abandoned land and agricultural use. The forest areas are mainly pine trees and areas with Mediterranean underbrush containing a mix of oak and pine (*Q. ilex* and *P. halepensis*). Site ES2 (Fig. 3B) is located near the town of Casasana in the province of Guadalajara 130 km northeast of Madrid. The surrounding relief is hilly and the site is undulating with a gentle slope. The mean annual temperature and precipitation is 14 °C and 457 mm, respectively. The lithological substrate is mainly formed of Miocene clays, marls and white sand. The soils have a silty clay loam texture with a pH of 7.8 and an abundance of $CaCO_3$ of 22% with a presence of gypsum. The natural vegetation of the area is Mediterranean underbrush made up of oak (*Q. ilex* and *Q. faginea*) and poplar along streams (*Populus sp.*). In both test sites agricultural activity used to include: cereal crops (wheat, barley, oats), legumes (chickpea, bean, lentil), vineyards, olive groves, fruit trees (almond, walnut, cherry, apple, pear), hemp, sumac, melon and pasture for sheep and goats. However, due to low productivity of the land and diminished population in the rural areas after migration to the big cities in the sixties and seventies, vast stretches of land have been abandoned and become marginal lands. **Norway**. A field experiment was established at Særheim, Norway (58°46′N; 5° 39′E; about 90 m asl) in the autumn of 2016 on a site, which has been cultivated with variable intensity for about hundred years (Fig. 3C). The site has continuously received manure, in particularly large amounts during the last 50 years. The climate is oceanic with cool summers and mild winters, and an annual precipitation of approximately 1200 mm. A weather station is installed approximately 100 m from the experiment. The moraine soil of glacial origin at the site has an organic matter of approximately 7% and phosphorous content of approximately 5 mg/100 g. In addition to plots with the original soil, a glacial deposited soil/moraine sandy soil with low organic (approximately 1%) and nutrient content from a nearby site replaced the upper A-horizon soil layer (about 25 cm) on half of the experimental area. Timothy grass (*Phleum pratense*) (cv Grindstad) and tall fescue (*Festuca arundinacea*) (cv Swaj) were seeded at a rate of 35 kg ha^{-1} in September 2016. A complementary seeding was carried out on April 19, 2017 to ensure sufficient plant coverage. Four soil amendment treatments: 1) separated dry fraction pig manure, and mineral fertilizers, 2) separated dry fraction digestate from pig manure and mineral fertilizers, 3) mineral fertilizers and 4) biochar, separated dry fraction pig manure, and mineral fertilizer, were incorporated into the experimental soils before sowing. Each combination of soil, grass species and amendment was replicated four times on plots with an area of 3 m × 7 m. Soils physical properties and nutritive content were analyzed at the establishment of the experiment. Soil samples for analysis of soil microbial activity and functionality were taken at the same time. Soil nutrients and microbial activity and functionality will be analyzed at least yearly. Plant biomass, leaf area index biomass and quality variables will be measured repeatedly during 2017 and 2018. **France**. St Médard d'Eyrans (FR1): The wood preservation site (6 ha) is located in southwest France (Fig. 3D) nearby Bordeaux, and has been used for over a century to preserve and store timbers, posts, and utility poles (Mench and Bes, 2009). The industrial facility dates back to 1846. Creosote, Cu sulfate (from 1913 to 1980), CCA (from 1980 to 2006), and Cu hydroxycarbonates with benzylalkonium chlorides (since 2006) were used successively. Established vegetation and site characteristics are detailed in Bes et al. (2010). Anthropogenic soils are developed on an alluvial soil (Fluviosol, Eutric Gleysol). Soil investigation pits (0–1.5 m) revealed major contamination of topsoils by Cu and its spatial variation (65 to 2400 mg Cu kg^{-1} soil DW) whereas total As and Cr, i.e., 10–53 mg As and 20–87 mg Cr kg^{-1} in topsoils, were relatively low in all soil layers. Several phytomanagement options, i.e. high yielding crops (sunflower–tobacco crop rotation, barley), short-rotation coppice (willows, poplar, and false indigo), Miscanthus, vetiver, and mixed tree stands (poplar/scots pine; *Cytisus striatus*/*Salix caprea*, *S. viminalis*). Soil amendments are assessed: compost and dolomitic limestone, alone and in combination, compost with iron grit, basic slags, biochar, compost pellet, separated dry fraction and dry fraction digestate from pig manure. Parc aux Angéliques (Chaban–Delmas and Borifer sub-sites, FR2): The Chaban–Delmas site (4.5 ha) is located in southwest France (Table 2), in Bordeaux downtown, at the outlet of the Chaban–Delmas bridge, on the right bank of the Garonne River. This former harbor dock is a brownfield site. From October 2009 to December 2012, it was used as a repository of material stocks and machinery required for the bridge construction. The Bordeaux city has decided to convert it into an urban park. The technosol developed over embankments displays a sandy texture with high total TE concentrations (in mg kg^{-1} DW; Zn [392–7899], Cd [1.7–9], Cu [140–2838], As [41–182], Pb [301–1306], and Ni [20–114]) and PAH concentrations (26–163 mg kg^{-1} DW) in soils exceeding the background values for French sandy soils, under alkaline conditions (pH > 8). Such soil contamination is the legacy of former industrial and harbor activities located on the Garonne riverbanks. Plots are phytomanaged with herbaceous plant species, i.e. alfalfa (*Medicago sativa*), ryegrass (*Lolium perenne*), *Bromus sterilis, Festuca pratensis*), alone and in combination with poplars (*Populus nigra*). Evin-Malmaison (FR3): Agricultural plots are located at Evin-Malmaison, at roughly 1 km from a former Pb/Zn smelter, Metaleurop Nord (Nsanganwimana et al., 2016). The site landscape is highly anthropized with residential suburbs, agricultural and woodlands, and transport networks (Fig. 3 D). The soil is a clay sandy loam dominated by silt (53%), and with a slightly alkaline pH. The total carbonate, organic carbon, total nitrogen, and P_2O_5 contents are higher in topsoil than in deep horizons. The soil metal contamination is restricted to ploughed horizon (0–30 cm). Topsoil is mainly contaminated by Cd, Pb, and Zn at concentrations (mg kg^{-1}) of 14.1 ± 1.4, 731 ± 67 and 1000 ± 88, respectively. These concentrations are 33, 23 and 15-fold (for Cd, Pb, and Zn, respectively) higher than regional background concentrations in uncontaminated agricultural topsoils (Sterckeman et al., 2002). Compost, either initial state or pelleted, and biochar were applied. Hemp was cultivated in 2017. **Germany**. Martlhof (784 m a.s.l.), a traditional small dairy farm, was founded in 2016 on former extensively used grassland between Tegernsee and Schliersee, next to the Alps (Fig. 3E). The mean annual precipitation in this region is 991 mm, the mean annual temperature 7.5 °C. The relief is gently sloping and the soils have a sandy loam texture with a pH ranging from 5.7 to 7.0. Martlhof is an ongoing small-scale farm aiming to increase its value creation by implementing aspects of circular bioeconomy. Besides producing fodder for dairy, pigs and horses, it operates a pyrolysis reactor to recycle plant residues and produce energy, heat and biochar. A fully randomized field plot with 48 different plots was implemented at Martlhof to study (a) the microbial diversity changes due to the conversion situation, (b) the health and performance of crops in unfertilized, and organically fertilized plots, and (c) the biomass production on the plots in comparison to the original grassland. In the first growing season, all plots were homogeneously fertilized with organic fertilizer (pig and sheep manure) and subsequently sown with *Vicia faba*, to equalize the initial soil situation. Crop rotation using maize, fodder beet, and barley, with *V. faba* as intercrop will be set up in Martlhof, with an additional group of *Miscanthus* plots (as permanent crop). Martlhof will utilize maize, beets and barley as fodder, and *Miscanthus* as energy crop and for biochar production. Results of a basic inventory on soil parameters show high homogeneity of the soils under the plots, but also differences in fertilization status due to overgrazing. Pelleted compost as well as digestates are used to fertilize this plot experiment. **Poland**. The experimental station of Skierniewice was founded in 2002 on the long-term fertilizer experiments of an experimental field from 1921. The mean annual precipitation in this region is 528 mm and the mean annual temperature 8.0 °C. Field I (Skierniewice) and Field II (Miedniewice) are covered with soils of glacial origin, on ground moraine. The dominant types of these soils are stagnic luvisols (about 90% of Field I and about 60% of Field II). The substratum is loamy sand (14–17% of silt) to a depth of 40 cm and loam in deeper soil layers with a low total organic carbon content of 0.6–0.75%. Field I covers an area of 27.83 ha, including 25 ha of arable land. Irrigation is needed because of the low water holding capacity and the low mean annual precipitation. Maize, Timothy grass and tall fescue are planted to examine effects of varied fertilization on crops and environment in different crop rotation systems (Fig. 3F). Different fertilizers including organic wastes (e.g. pelletized compost from spent mushroom substrate, bio-rest from biogas production and straw) are applied as soil amendments to discover differences in plant growth, biomass yield and microbial diversity. The on-site produced pellets are provided for some field experiments conducted in the INTENSE project. **Italy**. Azienda Agraria Sperimentale Stuard (Fig. 3G,H) is a small experimental farm sized 20 ha, operating since 1983, located in the upper Po valley, at the center of an alluvial substrate with varying weaving (from gravel to clay), put in place by significant flooding events related to the major watercourses of the area (Taro, Parma, Baganza). In the region, the mean annual temperature is 12.5 °C (ranging from −2 to 29 in 2016) and the mean annual precipitation is 842 mm. This is a relatively stable area, from historical times no longer affected by sediment yields, in which the soils have had time to differentiate significantly from the substrate of origin (medium-to-moderate tessitural floods). On the farm there is a moderate variability in soil characteristics mainly related to variations in the soil profile. The plot area is located in the central-western sectors of the farm, where soils have agronomic qualities mainly affected by high silt content. They are moderately alkaline and have superficial horizons, about 50 cm thick, of olive-brown colour, lime clay, very limestone and very deep, 30–70 cm thick, light brown olive, strongly calcareous. These soils fall into the utmost fine, mixed, mesic Ustochrepts according to the Soil Taxonomy and the Haplic Calcisols according to the FAO Legend. There are no significant physical limitations to the development of radical apparatus. The characteristics of the structural elements determine favorable conditions for the entire soil volume to be rooted. The presence of an ancient soil buried with features favorable to rooting allows plant roots to deepen without problems. Clay content, despite the high amount of silt that is always present, results in ties of sufficient intensity between the soil particles: The stability of the structure is generally good and crusts are formed only after intense rains. The randomized experimental plots are planted with maize rotated with barley and supplemented with biochar from wood material, compost as pellet, organic fertilizer (manure) and mineral fertilizers.

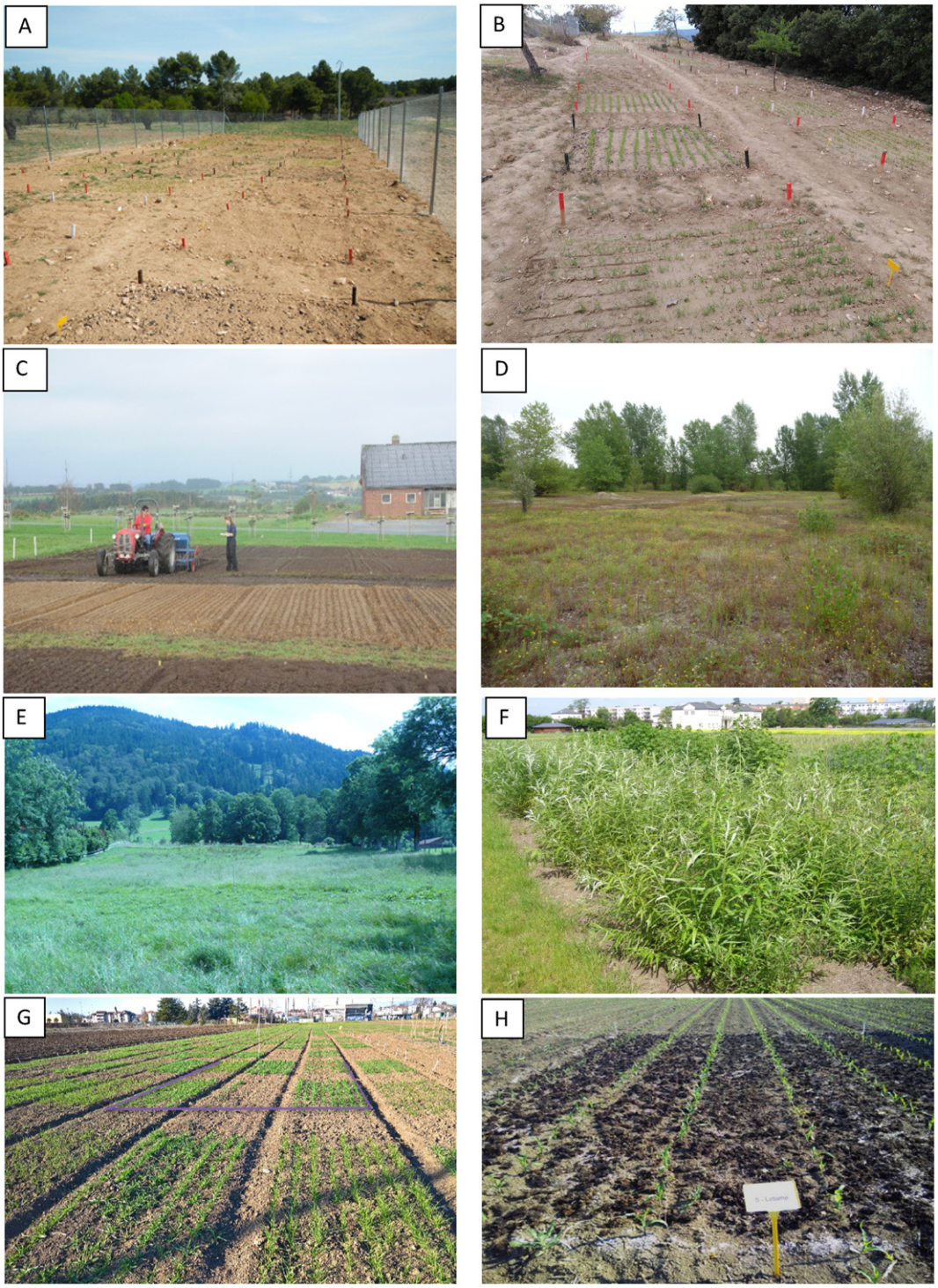
amending organic matter to soils will increase the aggregate formation potential, over-fertilization can lead to an uncoupling of processes that challenge the whole ecosystem and its productivity.

It becomes clear that anthropogenic activities cause soil quality losses over time, which may not revert easily. Failure to protect soils after disturbance results inevitably in their degradation will consequently have environmental impacts and affect other precious ecosystems and even human life. Hence, the primary objective of soil restoration must be to minimize further degradation and unbalanced nutrient losses. Mitigation technologies are urgently needed, effective both in decontaminating and in preserving soil quality and functions, including biodiversity. Emphasis should be on affordable costs and to promote the re-establishment of a functional plant-soil system for the long-term. Methods must aim at the natural rehabilitation potential of soils, integrating existing knowledge on soil resilience functions.

Given the large areas of land which both according to production, ecological and health criteria can be considered degraded, it is ever so important to develop and implement practices which aim at restoring the production potential in ecologically sound and sustainable ways.

3. Scenarios from an interdisciplinary project

In the Framework of the EU-FACCE JPI, the INTENSE project investigates test sites in France, Germany, Italy, Norway, Poland and Spain



(Fig. 2, Table 1). These sites represent problems associated to marginal soils and are characteristic for low productivity, water scarcity, or inappropriate landuse, others are prone to contamination by trace elements or organic pollutants. Their situation is complicated by the fact that mixed and multiple pollution occurs.

4. A toolbox to transform marginal land into productive land

4.1. Detecting the hotspots

Conventional farming of land has always involved homogeneous application of seeds, agrochemicals and mechanical methods. With increasing mechanization, larger farms and bigger machines, standard application practices according to the average soil characteristics on regional scale developed. However, farmers and land owners always knew from long term observation and site inspections that their land was not homogeneous at all, and that soil quality and yields differed strongly within fields. Indeed, when the first yield monitors were operated in the 1990s such differences in sections of arable fields could be documented in an exact manner (Schmidhalter et al., 2008). It was in fact a revolutionary step when spatially resolved soil information could be gained by electromagnetic induction, near-infrared spectroscopy, and indirectly by correlating spectral analyses of plant stands to soil properties. Using such an array of novel methods, characterization of soil texture, soil carbon, and plant available water in the soil improved tremendously. Determining relevant soil properties by contactless sensor techniques became highly effective and provided long-term information for optimized management. Even more, today remote and proximal sensing allows also determining plant biomass, nitrogen content, and nitrogen uptake, by that providing the basis for management decisions (Kyratzis et al., 2017). With new generation computers, data processing became easier and faster, and precision agriculture developed. This technology bundles IT based tools to account for the variability and uncertainty within agricultural production systems.

Computer based sowing, plantlet positioning followed by precise irrigation or agrochemical application completed the picture, however at increasing costs. Nevertheless, farmers express willingness to pay for these services (Vuolo et al., 2015). Of course, this may depend on the size of the farm and the return of investment for the land owner. Instead of investing in precision farming equipment themselves, farmers may rely on extension services providing them with the required information and tools. The EU has recently addressed the application of precision agriculture as an approach to sustainably intensify food production, achieving food safety and security (European Parliamentary Research Service, 2016). This will optimize the use of natural resources such as water and nutrients as well as to site- and culture-specific application of agrochemicals and will pave the way for tomorrow's integrated productivity.

Mobile proximal sensors and drones are emerging technologies designed to overcome many of the limitations associated with current use of satellite- or aircraft-borne sensing systems for mapping crop condition and soil quality in arable land. Recent advances in optical designs and electronic circuits have allowed the development of multispectral proximal sensors. The polychromatic bank of light emitting diodes (LEDs) emits light in three wavebands: red, red-edge and near infrared (NIR). The NIR:red ratio is sensitive in detecting water stress of canopies, while the red:red-edge ratio is sensitive to chlorophyll content and consequently, to nitrogen deficiencies (SPAD, Olfs et al., 2005). Similarly, soil humidity sensors based on conductivity (EM38) are also in use (Heil and Schmidhalter, 2017).

When site management is assisted by such multi-parameter measurements of the status of soils and plants, datasets can be integrated and georeferenced to support decision making. Taking into account that factors affecting crop yield are so complex that even elaborate statistical methods can only give improved, but never accurate results, fuzzy logic approaches are more and more replacing older models in agriculture (Papageorgiou et al., 2011). Utilizing tools of precision

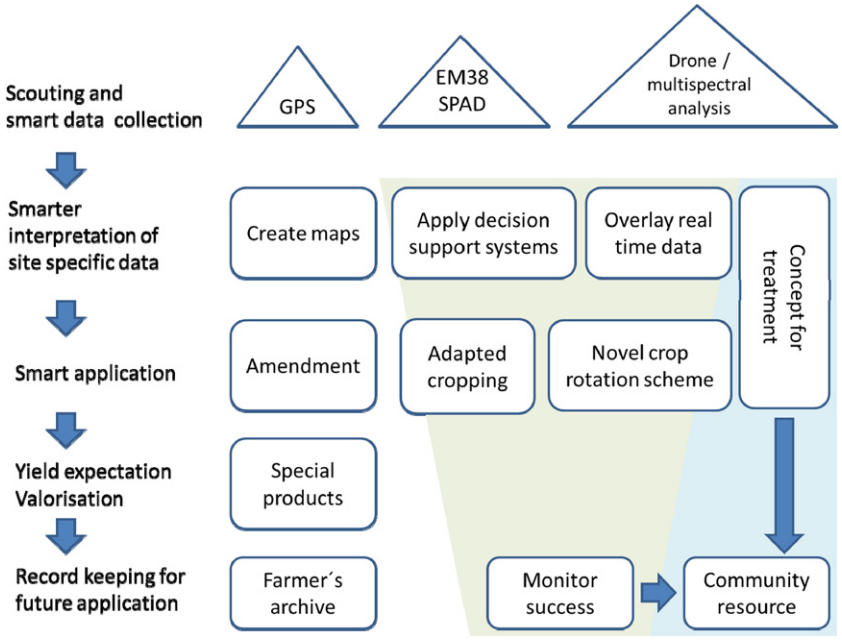


Fig. 4. Using precise tools for management of marginal land. Derived from high-tech precision agriculture solutions, modern sensors allow farmers to obtain a better knowledge about sites, their land, soils and their crops. To date, spatial collection systems are in use for collecting georeferenced data by making use of hand held (SPAD) or vehicle-borne (EM38) sensors and measuring devices that send wireless data to a managing unit. Remote sensing with satellites or airborne vehicles (e.g. UAVs - unmanned aerial vehicles, Zhang and Kovacs, 2012) and proximal on-field sensing attached to agricultural machines can be used to obtain hyperspectral imaging to monitor the physiological status of the vegetation (Morari et al., 2013; Pádua et al., 2017). Many presently available precision farming tools can be utilized to unlock the marginal soil's potential. Smart combination of methods is the key.

Fig. 5. Aerial picture of the experimental plot at the Martl-Hof, Bavaria, taken with an XR6 Drone and a Sony α6000 camera in RGB mode from 100 m distance. Crop types, quality of the grassland, animal distribution (right edge) and soil features can easily be distinguished (© PS).

agriculture is no longer cost intensive and time consuming. Nevertheless, they may require that the farmer adopts a different way to manage and treat the available land – from map creation to community support (Fig. 4).

Unfortunately, remote sensing is scarcely used for marginal lands (Gibbs and Salmon, 2015), although it would be of significant benefit if applied (Fig. 5). For plants grown on degraded land hyperspectral imageing can be used to determine soil degradation due to erosion (Schmid et al., 2016; Žížala et al., 2017), to identify plant stress due to leachate percolation from landfills (Ferrier et al., 2009), and pesticide contamination (Morari et al., 2013). Statistical methods like data fusion could help to optimize the outputs from tools mentioned above. Future scenarios must allow open and unbiased views on existing technologies, and options for their implementation. It would make a lot of sense to combine practices of integrated farming with ecological, organic and biological approaches, to gain moderate productivity while simultaneously protecting ecosystem services.

4.2. The role of amendments to increase long term productivity

Adding amendments to soils has been farming practice for generations, with the underlying idea that addition of external nutrients or structure building matter would improve soil fertility more or less immediately, or that soils were perfect sinks for (organic and inorganic) waste. This partial misinterpretation has led to countless smaller or bigger soil problems in agriculture and gardening, causing over-fertilization at best, but also salinization or soil destruction, before the faults of the oversimplified concept had been recognized. In itself, the addition of compost is a beneficial act, since it retains water and controls soil temperature, but, as we know today, it has to be properly planned with respect to sources, amounts and timing. In many conditions, especially sandy soils, the most effective methods of improving soil fertility relate to adding organic matter, by that increasing the capacity of the sorption complex, to retain more water in the rhizosphere (Table 2).

4.3. Compost qualities: Reducing pathogens by suppressive composts

The processing of waste organic matter is a common procedure. Almost 50% of the compost produced in Europe is used in agriculture (Sayen and Eder, 2014). With regards to compost qualities, it is important to consider nutrient composition and physical, chemical and physico-chemical properties, directly followed by the state of disease suppressiveness (pathogenic organism indicators). Both factor groups will be influenced by the degree of compost maturity and stability. Within EU Member States, standards for compost use and quality differ substantially, partly due to differences in soil policies (Table 3).

Sanitary properties are pivotal in evaluating the quality of composts. Across the EU the most common evaluation criteria are the contents of *Salmonella* and *E. coli*. Untreated composts prepared from waste organic matter may transfer microbiological risks, depending on the initial composition of the substrate. Application of immature composts may even increase pathogen populations. The addition of e.g. untreated sewage sludge probably increases the content of pathogens and the risk of crop failure or adverse health effects (Matei et al., 2016). In many EU countries, basic procedures are implemented to achieve hygienization, e.g. by raising the temperature during composting (Supplementary Table 1). In summary, fermentation processes should reach at least 55 °C for 24 h, and fermentation should not last < 12 days.

Besides, the quality of the compost and speed of the composting process is influenced by many factors (Supplement Table 1).

4.4. Municipal slurries

Municipal slurries may differ a lot in quality according to cleaning methodology and to which enterprises and product lines connect to the system. The content of metals should be monitored, even if plant availability may be low (Farrell and Jones, 2009). The treatment of slurry may imply methodology affecting the availability of certain nutrients, for example precipitation of phosphorus through use of $FeSO_4$ which may decrease availability of P to plants (Krogstad et al., 2005). The

Table 2
Ways to improve agricultural suitability of sandy soils permanently or temporarily dry.

Methods	Expected degree of improvement of the soil		
	High	Medium	Low
Addition of materials with high brevity (silt, clay, etc.).	X		
Addition of permanent organic matter such as biochar, brown coal	X		
Irrigation	X		
Construction of reservoirs of water		X	
Woodlots			X
Positive balance of organic matter			X

Table 3
Compost criteria for its qualification as product/waste in different European Member States. Compiled from Sayen and Eder (2014).

Country	Compost status	Criteria for the definition of compost status and its use on soil
Flanders (Belgium)	Product	Requirements on: Input materials; Process conditions; Product characteristics and use
Wallonia (Belgium)	Waste	Among the four classes (A–D) defined by the Government Decree, compost belong to class B and can be used on/in agricultural soil. Within class B, subclasses B1 and B2 are distinguished. The main difference lays in the acceptable metal content.
Germany	Waste	Requirements established by the bio-waste Ordinance. On a voluntary basis, if certified under the QAS of the RALGZ 251, compost can be put on the market and used as a product
Italy	Waste/Product	Requirements of the Legislative Decree 75/2010 must be fulfilled for compost use as fertilizer. If not, environmental restoration applications can be considered, when limit values of Inter-ministerial Decree 27/7/84 are fulfilled. Otherwise compost is considered as waste.
Poland	Waste/product	According to the Waste Law/Fertilizer Law
Spain	Product	Origin from specific input materials; – Documented life cycle (from waste reception to product selling); – Requirements for compost qualitative characterization.
Norway	Product	Application according to content of heavy metals, the plant's need for nutrients and the kind of products produced in the soil.

hygienization of slurry through use of large quantities of lime may increase the pH to very high levels and thus also limit nutrient availability. If enterprises on the slurry net have production that comprises use and leaching of metal(loid)s, then these compounds will follow the stream to the cleaning unit and will be carried to the final slurry. Another worry may be input of organic pollutants from both enterprises, from use and (inappropriate) disposal of pharmaceuticals from private households. Finally, the content of microorganisms should be monitored in municipal slurry. Prior to agricultural use of municipal slurry, the content of metal(loid)s, organic and inorganic pollutants should be checked, and safety guidelines tailored to different soils should be followed, to exclude potentially dangerous waste fractions from application to soils (Antonkiewicz et al., 2017). The responsibility for the slurry quality lies in the enterprises producing it, but the receiver should also have liabilities that the quality is what is to be expected. Lack of analysis methodology for all problematic compounds may be a problem related to municipal slurry. For many types of municipal slurry, the same quality criteria as for compost apply.

4.5. Utilize manure/digestate from biogas production

Besides adding plant residues, recycling of animal manure is a well-established method to provide nutrients to agricultural crops. For centuries, the combination of crop and animal production has been vital to maintain soil fertility and uphold plant production. However, the introduction of synthetically produced plant fertilizers meant that supply of farm manure was not anymore a prerequisite for successful crop production (Schröder, 2005). Under the pressure of animal husbandry for meat production, immense amounts of manure are produced that have to be managed, e.g. by spreading it on fields for intensive crop production. At the best, the produced crop biomass will be fed to the animals, by this approaching a closed system. Adequately handled manure can increase soil organic matter, water holding capacity and improve other soil physical properties such as infiltration capacity and hydraulic conductivity (Haynes and Naidu, 1998). Efficient recycling of manure could reduce the need of mineral nitrogen fertilizers whose industrial production requires large amounts of energy frequently supplied by fossil sources (Fischedick et al., 2014) and mineral phosphorous fertilizers which is a limited resource, even though estimated world phosphorous reserves have increased during the last years (Scholz et al., 2013).

Since our current understanding of soil processes has greatly moved forward, there has been a clear focus on improving the recycling of manure as plant fertilizer during the last decades. Several studies show the benefits of manure application on soil microbial activity and functionality under a wide range of conditions (see chapter 5). Field experiments showed a higher soil microbial biomass after application of organic manure than after application of non-organic fertilizers or no fertilizer application (Peacock et al., 2001; Chu et al., 2007; Liu et al., 2010). In addition, field experiments have shown that manure affects microbial community composition (Peacock et al., 2001) soil enzyme activity (Liu et al., 2010) and catabolic substrate utilization profile (Sradnick et al., 2013) more than ammonium nitrate or no fertilizer application. However, despite beneficial effects of manure on resource use efficiency and soil productivity, manure application can sometimes impose stress to the environment. In manure and other organic substrates the nutrients are largely bound to compounds that cannot be taken up easily by plants. Thus, their efficient use requires that nutrient availability is synchronized with plant nutrient demand and climatic conditions that favor nutrient uptake in roots. If manure applications are not synchronized, risk of losses of nutrients to the environment is large, notably for nitrogen through ammonia volatilization, denitrification and nitrate leaching through surface runoff and drainage water processes. Besides resulting in an inefficient resource use, nutrient losses can contribute to climate change, depletion of the ozone layer, eutrophication and acidification (Cameron et al., 2013). Other risks to the environment associated with manure are the spread of antibiotic resistant bacteria (Heuer et al., 2011) and metal(loid)s (Dach and Starmans, 2005). In addition, manure application to crops with heavy machinery can easily cause soil compaction and entail negative effects on soil physics, biological properties and plant growth (Nawaz et al., 2013).

The production of bioenergy may partly decrease the dilemma of overloads. Anaerobic digestion (AD) of manure and other organic feedstocks may be used to generate methane, replacing fossil energy. Energy production through AD has increased rapidly during the last years, especially in farm scale facilities, also due to EU subsidies (Mao et al., 2015). The rest product from AD, digestate, is suitable as fertilizer due to its high content of nutrients (Möller and Müller, 2012). Although digestate composition is related to the feedstock that is digested, the AD process changes its physical and chemical properties. Typically, during AD the manure undergoes an increase in pH and ammonium nitrogen as the share of the total N, lower organic matter and C/N ratio, and lower biological oxygen demand (Möller and Müller, 2012).

Similar to manure, digestate has a positive influence on soil microbial activity and biomass (Chen et al., 2012; García-Sánchez et al., 2015a), indicated by beneficial effects on soil functionality. While differences in soil microbial community and activity between manure and digestate were not such that they justified the recommendation of either substrate before the other (Abubaker et al., 2013), Insam et al. (2015) concluded that digestate could enhance soil microbial activity and biomass as compared to manure. Similar to manure, the high proportion of unavailable organically bound nutrients in digestate require scheduled applications, synchronized with plant nutrient demands. However, the higher share of ammonium nitrogen in digestate means that a larger share of the nitrogen is directly available to plants (Cavalli et al., 2016). Accordingly, digestate has also a higher ammonia and nitrogen emission potential than undigested manure (Nkoa, 2014). Moreover,

experimental studies show that the concentration of nitrate in upper soil layers is higher after application of digestate than after manure application (Goberna et al., 2011).

Digestate, especially when processed from pig or chicken manure, contains higher amounts of metal(loid)s than manure (Demirel et al., 2013; Zhu et al., 2014), which suggests that its application could be a concern, particularly on those soils which already contain trace elements. However, other studies found smaller amounts of metal(-loid)s in digestate from poultry manure than in digestate from energy crops (Lehtomäki and Björnsson, 2006) or food and garden waste (Govasmark et al., 2011). In any case, application of digestate may help immobilize metal(loid)s in soils where they occur in high concentrations (García-Sánchez et al., 2015b).

There are techniques to separate manure and digestate into nitrogen and potassium rich liquid and a phosphorus rich solid phase to facilitate its recycling and adapt its nutrient content to the specific demands of different crop and nutrient status (Möller and Müller, 2012). The solid phase can be dried further and/or pelleted to decrease transportation costs. The liquid phase can be applied using traditional or sophisticated techniques. Exploration of such tailoring could provide useful knowledge about the effects of digestate and manure application on soil microbes to set efficient application regimes and techniques.

4.6. Adding biochar to soils

Biochar is a recent addition to the list of agricultural amendments but the use of charcoal in soils in truth dates back thousands of years (Qambrani et al., 2017). Biochar is the solid product derived from waste biomass pyrolysis, under mid to low oxygen supply and high temperatures (Lehmann et al., 2011; Ahmad et al., 2014). Still, research on it is in its infancy. Currently, char or biochar is produced from the pyrolysis of plant biomass and other kinds of waste of plant or animal origin: applications of biochar resulting from energy production contributes to close the production cycles, and its proposed efficacy as adsorbent and amendment may increase environmental sustainability and cost effectiveness. Hence, the properties and applications of biochar must also take properties of the feeding material into account. The main role of biochar is in carbon sequestration, with carbon representing up to 90% of the mass, thereby contributing to mitigation of greenhouse gas emission and climate change. Even though carbon in char is considered stable and not bioavailable, its application to soils can increase soil fertility mainly through positive effects on soil structure and functionality (Agegnehu et al., 2017). Containing pores and internal surfaces, depending on the structure of the starting material, biochar confers interesting features for amendments, modifying the Cation Exchange Capacity (CEC) and Electric Conductivity (EC): use of biochar was shown to increase soil water retention and availability of some nutrients to plants. While larger amounts of biochar could exert negative effects on plant growth, the co-application with manure fertilizers seems to decrease those negative effects (Ippolito et al., 2015). Biochar can limit translocation of non-essential elements to plants (Beesley and Marmiroli, 2011; Beesley et al., 2013; Oustriere et al., 2017), effectively contributing to canopy tolerance towards organic and inorganic contaminants. It may also stimulate microbial communities able to degrade xenobiotics (Rizwan et al., 2016) and it can reduce leaching and phytoavailability of trace elements (TE) in contaminated soils (Park et al., 2011). However, all these potential gains depend on its quality (Oustriere et al., 2016). At the same time, it can boost plant defense against biotic stresses, and pathogen attacks. Having a microstructure with pores of different dimensions and functional groups exposed on the surfaces, biochar can be favorable to microbial colonization, and this in turn has beneficial effects on soil fertility (Lehmann et al., 2011). Hence, innovative applications foresee functionalization of biochar with beneficial microorganisms to decrease the use of chemical fertilizers. Biochar made from the solid fractions of manure and municipal wastes, after separating out the N-rich liquid fraction, may be most valuable as fertilizer and soil amendment. The phosphorus supply was improved when Jin et al. (2016) tested P-effect of manure char in clay and silt soils. The better use of nutrients in circulation will decrease the climate footprint of chemical fertilizer production and contribute to closing gaps in the circular bioeconomy, also, since it starts from waste material and it produces energy and biofuels.

A main issue with biochar is the need for standardization of requirements for distribution and harmonization of analytical procedures. Efforts in this direction have been performed by the European Biochar Certificate; it is now considered by the "Voluntary Carbon Standard Program" in the framework of agricultural practices contributing to carbon sequestration.

4.7. Lower fertilizer inputs, sustainable and economically feasible methods

To date, increased production of fertilizers and soil fertilization contrasts with a relatively low nutrient assimilation by crops. On average, the uptake of fertilizer nitrogen by plants is about 50% of the available N on site, and it is estimated that assimilation of phosphorous is about 10–25% and potassium reaches 50–60% of the applied amounts. This discrepancy leads to an environmental dispersion of excess mineral nutrients that will not be completely used up during plant production (Lubkowski, 2016).

During the industrial production of mineral nitrogen fertilizers, also climate gases and waste are emitted (Fischedick et al., 2014), and mineral phosphorus production relies on non-renewable limited sources (Scholz et al., 2013). One method of limiting the adverse effects would be adjusting fertilizer inputs in crop production.

Reducing the amount of mineral fertilizer can be achieved by either increasing the fertilizer nutrient use efficiency or by replacing mineral fertilization by organic amendments (Fig. 6). Fertilizer use efficiency can be optimized by best management practices applying nutrients at correct rate, time, and place - accompanied by adequate agronomic practices (Johnston and Bruulsemab, 2014).

Selecting the right source – it is pivotal to select the right source of fertilizer for achieving individual goals that will meet specific economic, environmental, and social objectives at a given site.

Setting the right rate: The fertilizer requirements vary depending on the type of soil and plants. Therefore, the amount should be determined on the basis of soil testing, i.e. once every four years. Over- or under-application will result in reduced nutrient use efficiency or losses in yield and quality.

Choosing the right time: Fertilizer should be applied during the growing season so that the plants can take up the required amount of nutrients. It should never be applied when soil temperatures are in the range of 0–6 °C or to any substrate above its field capacity.

Determining the right place: Biogenic components (nitrogen and phosphorus) should be used in accordance with the principles of good agricultural practice especially in sensitive areas (Johnston and Bruulsemab, 2014), and following a mapping approach (see Fig. 4).

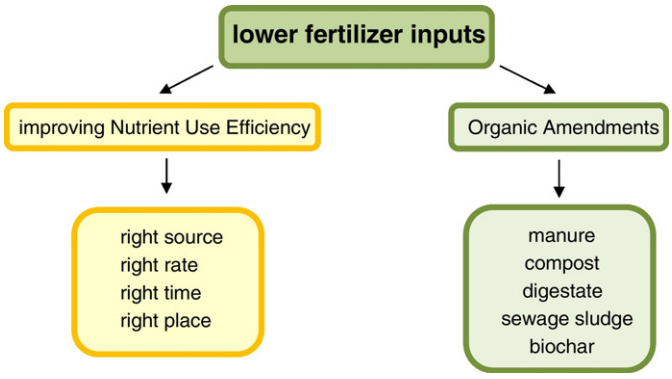


Fig. 6. Methods to reduce the fertilizer input.

Table 4
Availibility of different kinds of urban organic wastes in different European countries.

Country	Sources [Mg year^{-1}]		Fertilizer amounts produced	
	Green wastes	Household bio-wastes	Composts	Digestates
Germany	5,000,000	4,000,000	5,000,000	430,000
Norway	160,000	250,000	112,000	45,000
Poland	549,400	1,896,000	1,154,000	2,000,000[a]

[a] Digestates from agriculture biogas plants.

Precision agriculture methods like phenotyping with unmanned aerial vehicles (UAV) can help in this respect.

Reducing the consumption of mineral fertilizers can also be achieved by using waste organic substances (Table 4). About 32% of composts originates from biowaste and 9% from mixed waste, whereas the remaining part derives from sewage sludge and green waste (Sayen and Eder, 2014).

Organic amendments, in particular compost, can represent a valuable tool to improve soil fertility sustainably, since they contain all nutrients required for crop growth. Applying these amendments in marginal soils will positively influence a number of soil properties like soil organic carbon, available forms of phosphorus and potassium, microbial activity, water storage and soil pH. Of course, application of organic amendments will also improve soil structure. The use of such amendments is particularly important in sandy soils, which are characterized by poor water retention and physico-chemical properties, as well as rendzina soils.

Table 5 summarizes the main properties of amendments, highlighting the respective advantages and drawbacks. Sustainable agriculture in the future, as Conservative agriculture (CA), or as Climate-smart agriculture (CSA), will exploit all possibilities offered by the specific territory to obtain the maximum benefits from the soil amendments available, in order to recycle and reuse all kinds of agrofood residues and close gaps to reach a circular economy. At the same time, Table 5 highlights gaps in knowledge that must be filled in with basic and applied research.

4.8. A special case: Biological methods for soil remediation

When land is polluted by historical or recent industrial activities or contaminant spills, action has to be taken. Soil contamination due to

Table 5
Relevant properties of main categories of organic amendments as reported in literature (updated January 2017). Green and orange colour indicates positive and negative effects respectively; yellow colour indicates presence of both positive and negative effects; grey colour indicates lack of knowledge.

Properties	COMPOST[1]	ANIMAL MANURE[2]	DIGESTATE (anaerobic digestion)[3]	BIOCHAR[4]
Increase in content of organic matter	increases soil organic matter, humic substances	increases soil organic matter, depends on animal diet	depends on feedstock - humic acids (mainly solid fraction)	affects the stability of existing organic matter
Modification of C:N ratio			low C/N ratio due to digestion	increase
Improvement of water holding capacity	Increases		improves	increases due to surface structure
Supply of nutrients (N, P, etc.) nutrient balance	enhances nutrient supply	leaching of N and P – content differs with animal species	depends on feedstock - mineral N, P (mainly liquid fraction), possible leaching	reduces leaching of nutrients / slow release fertilizer - provides P and K
Modify pH	lowers pH		high pH	increase in soil pH of acidic soils
Modification of cation exchange capacity	Increases			increase in soils with low CEC
Improvement of texture and aggregation state	amelioration of structure and porosity	reduces density	reduces density, increase in aggregate stability	increase in porosity, stability of aggregates
Sequestration of pollutants/contaminants	through humic substances		not reported	can sequester pollutants, but also increase mobility
Addition of pollutants/contaminants	might contain persistent pollutants	micronutrients supplied to animals	might contain persistent pollutants, metals	can contain pollutants, in this case it is not usable
Decrease in salinity	Improvement		can increase salinity with repeated applications	can sequester salts and modify CEC
Soil conservation (e.g. minimise erosion)	remediates degraded soils		still to be investigated	still to be investigated
Increase in microbial biomass	increase	Increase	considerable increase	increase
Increase in microbial diversity	increase or decrease	Increase	significant changes	significant differences
Stimulation of specific microorganisms	no indication	antibiotic resistance	dominance of slowly growing microorganisms	arbuscular and ectomycorrhiza
Increase in enzymatic activities	increase in soil microbial activity	Increase	nitrogen mineralization, other enzymes	reports on increase in enzymatic activities
Increase in diversity of fauna	Limited observations, differing effects		limited observation, increase	Limited observations, differing effects
Effects on plants growth	positive	very positive	positive	mostly positive
Increase of yield	Positive	Positive	fertilizer capacity	reports on increase of crop yield
Increase of product quality	not significant			not assessed
Improve in defense against pathogens	Positive effects			Limited observations, positive effects
Origin, raw materials	biomass from different sources		biomass from different sources	biomass from different sources
Production requirements	requires large amounts of energy, long time			depends on biomass feedstock - importance of temperature
Standardisation of product	Quality assessment differs in the countries	not possible	not possible	just starting
Cost (including transport)	moderate		depends on feedstock	depends on feedstock - high
Positive carbon emission	emissions during composting	emissions of CH_4 and N_2O, NH_3	during digestion GHG emissions, NH_3 emission	could stimulate CO_2 emissions by microbes
Negative carbon emission	carbon sequestration in humic substances		decrease of emissions from manure	removal during growth of biomass, C - sequestration
Legislation, norms on applicability	Differences among countries		can be amendment or fertilizer	limited
Social acceptability	well established	well established	Low	not yet tested
Additional benefits (e.g. energy production)	scalable to farm		production of biogas	reduction of N_2O emissions
Ecosystem services of relevance				

[1] Martinez-Blanco et al., 2013; Cesaro et al., 2015; Medina et al., 2015.
[2] He et al., 2016; Bernal et al., 2009.
[3] Nkoa, 2014; Möller, 2015.
[4] Jeffery et al., 2011; Lehmann et al., 2011; Laghari et al., 2016; Tammeorg et al., 2017.

Table 6
Technologies for soil remediation. Typically, physical, chemical or biological methods may be applied.

Technologies	
„Ex-situ"	„In-situ"
Physical methods	
Incineration	Aeration
Thermal desorption	Soil vapour extraction thermally enhanced
Soil vapour extraction	Electro reclamation
Magnetic segregation of radioactive soil	
Chemical methods	
Soil washing	Soil flushing
Solidification/stabilization/sorption/immobilization	Solidification/stabilization/sorption/chemical immobilization
Dehalogenation	
Solvent extraction	
Chemical and photochemical oxidation/reduction	
Biological methods	
Composting	Bioremediation
Bioreactors/microbiological filters	Phytoremediation
Landfarming	Landfarming
Biopiles	Natural attenuation

metal(loid)s in excess, other inorganic contaminants and persistent organic chemicals are of particular concern (Mench et al., 2009, 2010). Contamination can seriously affect a soil's ability to perform its key functions in the ecosystem. Remediation is considered as the management of the contaminant at a site so as to prevent, minimize or mitigate damage to human health, property or the environment, including removal. A scheme depicting different methodologies for remediation is presented in Supplementary Fig. 1. Using site-specific precision technologies in plant nutrition can support both soil conservation and soil fertility maintenance (Németh, 2006). In any case, the aim of remediation is to reduce existing or potential environmental risks, to analyze and assess health and environmental risks to related pollution in the area, and to reduce the risk to a level that guarantees the return of contaminated sites into use as planned (Table 6). Phytoremediation with living plants (or plant-microbe associations) provides a set of options suitable for in situ and ex situ remediation of contaminated soils, sludges, sediments and ground waters through contaminant removal, degradation, sequestration, volatilization or stabilization (Marmiroli and McCutcheon, 2003). It can be used to remove or dissipate various contaminants including trace elements, pesticides, solvents, explosives, petroleum hydrocarbons, polycyclic aromatic hydrocarbons and landfill leachates (Vaněk and Schwitzguébel, 2003; Mench et al., 2003, 2006; Reeves and Baker, 2000; Schwitzguébel et al., 2002; Van der Lelie et al., 2001). Phytoremediation has been used for point and non-point source hazardous waste control. It received a great deal of attention from regulators, consultants, responsible parties, and stakeholders, and became an attractive alternative to other clean up technologies due to its relatively low cost, potential effectiveness and the inherently aesthetic nature of using plants to clean up contaminated sites (Marmiroli and McCutcheon, 2003). The accumulation of contaminants in the plants may present a problem with contaminants entering the food chain (e.g. herbivores) or cause the plants to become a waste disposal issue. Consequently, the relative concentrations of contaminants in the plant tissue must be determined, and proper harvest and disposal methods must be developed and approved by regulatory agencies. One option is to valorize the plant biomass to face energy and global change problems, e.g. by supercritical gasification, liquefaction and pyrolysis as potential routes. The first process results in the formation of syngas to produce e.g. heat or electricity, while the other processes lead to biofuel, biochar or valuable chemicals. However, the feasibility of such options is still in its infancy. When digestate contains too high trace element concentration for commercial fertilizers, pyrolysis may be an alternative. During pyrolysis mineral elements are concentrated in the solid fraction (sand and char). This may open possibilities for trace element recovery from this fraction, or when metal recovery seems not feasible, they are at least concentrated in only a very small mass fraction (needing to be disposed) compared to the initial biomass amount. Smart use of plant-microbe combinations can be applied to metabolize even highly recalcitrant organic chemicals with hazard potential (Sauvêtre and Schröder, 2015, Sauvêtre et al., 2017).

5. The role of crops on marginal soils

Crop rotation has been practiced since the middle ages as a result of population growth, land shortage and economic pressure and to counteract decreases in soil fertility. After World War II it was replaced by more intensive farming practices with mineral fertilizers, pesticides and new technologies to enhance yield (Tilman et al., 2002). Especially in Northern Europe cereal-based, intensive cropping was used instead of the more balanced cereal-legume-tuber crop rotation that had formerly been applied. Only in the last decades a change in farming management occurred with focus on ecology and sustainability: it has been rediscovered that abandoning crop rotation resulted in soil fertility decline (FAO, 1993) and increases soil erosion. With the cultivation of legumes, crop rotation reverts land degradation, increases soil fertility and enhances nitrogen availability. Another beneficial aspect is the regulation of weeds and disease suppression (Garrison et al., 2014). However, crop rotation is location-based and therefore ecological and economical aspects for regional stakeholders must be considered. Decision support systems with regard to cultivation order, demands for life stock farming or non-food crops for special purposes are required (Castell et al., 2015). In the context of increasing soil resilience, the C/N ratio is pivotal for an elaborate life cycle assessment of crop rotation schemes on the farm level.

5.1. Crop rotation schemes for derelict soils

Especially on marginal lands crop rotation can increase sustainability and lead to productivity. Typical crop rotation schemes in temperate regions should contain legumes (mulch or cut) – tuber crops – winter cereal – spring cereal. Undersowing of leguminous species has been proven to be beneficial (Schröder et al., 2008a). On richer soils with higher potential of soil erosion the direct sowing of grass or other lay crops after maize harvest could avoid erosion effects. Since enhanced grass silage amounts in mulch lead to extended biomass decomposition, a higher C/N ratio can be observed and therefore N immobilization is higher (Sainju et al., 2006). Some options for crop rotations on problematic soils are summarized in Table 7.

Eco-efficiency could be improved by exchanging cultivars which are dependent on higher fertilization rates with cultivars less dependent to

Table 7
Examples for crop rotations on marginal soils.

Soil type	Problems/conditions	Rotational scheme	Literature
Sandy soil	Low soil pH (5.5–5.8) Low soil organic matter (SOM) High soil irrigation demand	Cooksfoot (mulch or cut) – potatoes - winter wheat – oilseed rape - winter rye	Trost et al., 2014
	Low soil fertility	Oats – winter rye- winter barley – spring barley	Ellmer, 2008
Dry land (Great Plains)	Limited water Cold weather	spring wheat- lentil	Sainju et al., 2006
Thin black Cernozem	Poor grassland, cold weather, ineffective oilseed production	spring wheat–spring wheat–flax–winter wheat spring wheat–flax–winter wheat–field pea	Zentner et al., 2004
Bavarian Tertiary hills (e.g. Scheyern)	Erosion, compaction, intensive agriculture	clover/grass-potatoes-winter wheat-sunflower-clover/grass-winter wheat-winter rye, all with lucerne/clover undersowing	Schröder et al., 2008*a*
Bavarian Tertiary hills (e.g. Roggenstein)	Erosion, compaction, intensive agriculture – focus on energy plants	Giant wheatgrass – maize/winter wheat – grass legumes. Additional cultures of: Cup plant, Miscanthus, willow, poplar	Chmelikova, personal comm.

enhance output from the same rate of natural resources. Solutions that create higher yield and in parallel do not enhance environmental impacts per se have to be selected (Kulak et al., 2013). The aim is to maintain good ecosystem-services under unchanged yield demand and to preserve the quality of plant products for food and fodder, and even their biofortification (Jablonowski et al., 2017). Therefore crop rotation could enhance yield in low- input cropping systems without increasing environmental burdens, at the same time reducing crop–specific pathogens and taking advantage of symbiotic and biological nitrogen fixation (Kulak et al., 2013).

5.2. Plants for the removal of pollutants from contaminated soils

Selection of plant species and optimization of growth in the presence of contaminants are key players in successful "phytomanagement" of degraded and contaminated soils under different pedo-climatic conditions. Plants must tolerate numerous abiotic and biotic cues, e.g. water stress, soil acidity or salinity, nutrient deficiency, frost, soil erosion or compaction, herbivory, pests. In addition, for the gentle remediation options (GRO), they must at the same time tolerate any soil contaminant(s) present (Supplement Fig. 1). Of course, the first choice of plant genotypes is pioneer vegetation colonizing natural serpentine soils, present in surrounding areas, or established on metal-enriched substrates, such as ultramafic or calamine soils (Kidd et al., 2015). Regarding plant community development at trace element (TE)-contaminated sites, abiotic factors can be more limiting than competitive interactions between species (Che-Castaldo and Inouye, 2015). Within the same plant species various ecotypes, cultivars/varieties or clones can differ greatly in their response to the presence of contaminants (Vyslouzilova et al., 2003; Marmiroli et al., 2011; Ruttens et al., 2011; Kidd et al., 2015). To prevent spreading of the TE pollution, it will be important to stimulate microbial processes that could contribute to the phytostabilization of TE in the rhizosphere (Lebeau et al., 2008). The selection of endophytic bacteria and rhizobacteria for enhancing biomass production and quality on TE- and mixed contaminated soils is a current challenge (Janssen et al., 2015; Mesa et al., 2017). Intercropping can be an option to facilitate the phytomanagement of TE-contaminated soils, and plant densities as well (Deng et al., 2016; Bani et al., 2015), notably to phytoextract TE without affecting the productivity and quality of undersown legumes. Additionally, phytomanagement of contaminated soils can promote the structural and functional biodiversity within soil microbial communities (Cavani et al., 2016; Foulon et al., 2016; Touceda-González et al., 2017a, b), mesofauna (De Vaufleury et al., 2013), butterflies (Mulder and Breure, 2006) and other animals.

Organic pollutants pose a number of different challenges, however spill sites are manifold and pollutant uptake may be significant through root and foliar exposure. One major aim must be to prevent a pollutant plume from moving into groundwater or from spreading into so far unaffected regions of the soil. Using plants with high transpiration rates may be advantageous in this case. A second aim would be the accumulation of organics in the plant rhizosphere, for stimulating microbial activity and xenobiotic rhizodegradation (Taghavi et al., 2005; Barac et al., 2004; Weyens et al., 2009b). Macroporous trees and shrubs can prevent pollutant spread, and mixed plantations of species with different rooting depths might be capable to control the movement of pollutants in the soil (Schröder and Collins, 2002). Few species can take up lipophilic pollutants deliberately from the soil. In most cases, penetration is limited to the rhizodermis, i.e. the outer parts of the roots, which can be reached by diffusion. Transfer of PAH to shoots and leaves seems possible in *Cucurbitaceae*, i.e. cucumbers, zucchini and melons, whereas in plants like carrots, the compounds remain in the roots.

If, however, xenobiotics are metabolized, e.g. by hydroxylating or peroxidizing enzymes, in the root and the rhizosphere, the situation changes, and xenobiotics may well be able to enter the plant. Transfer through the plant has been demonstrated for many compounds (Cui et al., 2015, Chen et al., 2016). A bioremediation strategy for soils co-contaminated with Cd, DDT, and its metabolites was developed using the Cd-hyperaccumulator *Sedum alfredii* and DDT-degrading microbes (Zhu et al., 2012). In this case the question remains how effective the pollutant can be further degraded by the species of interest. From a practical point of view it would always be better to digest the plant material for bioenergy production, and safely dispose of rest fractions. In any case, be it organic pollution or excess availability of trace elements, harvested biomass should not be utilized as sources for food or feed.

6. Going underground: Exploiting microbe-plant interaction to strengthen plant health and production

As pointed out above, agricultural management strategies utilizing soil amendments such as compost and biochar mainly seek to improve soil fertility and the underlying ecosystem services by adjusting soil pH and increasing soil nutrient content and retention capacity (Diacono and Montemurro, 2010, Touceda-González et al., 2017a, b). Besides, soil amendments may also change microbial community composition and abundance, which in turn may influence nutrient cycles and soil structure, consequently affecting plant growth. In most soils amended with compost and other raw organic materials, microbiological activity and growth are stimulated as measured by microbial biomass C, basal respiration measurements and the activity of specific enzymes such as ureases and alkaline phosphatases (Diacono and Montemurro, 2010). In contrast to mineral fertilizers, slow and continuous release of nutrients from degrading compost will support microbial biomass for longer periods of time (Murphy et al., 2007). Similarly, biochars mainly derived from wood and cellulosic materials will stimulate bacteria and mycorrhizal fungi (arbuscular and ectomycorrhizal) by increased nutrient and carbon availability, decreased susceptibility to leaching through adhesion to the biochar, protection against competitors and predators, sorption of toxins and increased resistance against desiccation

(Lehmann et al., 2011). Therefore, both biochar and compost amendments appear a good option to foster the activity of beneficial plant-associated microorganisms.

6.1. General mechanisms of beneficial plant-associated microorganisms in plant growth

6.1.1. Nutrient cycling and soil nutrient bioavailability

The most prominent impact of microorganisms on soil fertility is their effect on nutrient cycles by fixing or mineralizing nutrients from the gross soil nutrient pool, making them available as biofertilizers (Hayat et al., 2010; Bulgarelli et al., 2013). Well-known mechanisms to promote nutrient availability include (a) biological nitrogen fixation whereby atmospheric N_2 is converted by bacterial nitrogenase activity into ammonia (NH_3) by symbiotic N_2-fixing bacteria and free-living heterotrophic bacteria (Dixon and Kahn, 2004); (b) nitrogen mineralization by fungi. Mycorrhizal fungi are especially beneficial for plants due to their ability to convert soil organic N into ammonium, which is partly shared with the plant host. To do so, they rely on proteases and chitinases specifically targeting major soil N sources: peptides and chitin (Chalot and Brun, 1998). When acting in concert with oxidative mechanisms this process improves the access to organic N from a polysaccharide-polyphenol matrix (Shah et al., 2015). (c) Phosphorus solubilization, whereby insoluble organic and inorganic phosphates (approximately 95% of the soil phosphorus) are transformed into plant-accessible HPO_4^{-2} and $H_2PO_4^{-1}$ through microbial production of organic acids (e.g. oxalate) and enzymatic mineralization (e.g. phosphatases) (Rodríguez and Fraga, 1999). And finally (d) iron solubilization, whereby inaccessible ferric ions (Fe^{3+}), which are dominant in the soil nutrient pool, can be mobilized through the production of low-molecular-weight iron-chelating siderophores by both monocots and microorganisms, thus improving iron bioavailability and uptake by roots and microbes (Wandersman and Delepelaire, 2004; Jeong and Guerinot, 2009). So far, broad-scale inoculation with specific microbes has been limited to nitrogen fixation and mineralization in greenhouse and field studies with sugarcane, rice and wheat (Hayat et al., 2010). Biological nitrogen fixation approximately accounts for 65% of the nitrogen currently utilized in agriculture (Weyens et al., 2009a, b).

6.1.2. Biosynthesis of phytohormones

Apart from their influence on the mineral cycle, plant-associated microbes can directly trigger plant health and growth through the biosynthesis of various signaling molecules, including homoserine-lactones (Sieper et al., 2013, Götz-Rösch et al., 2015) and phytohormones. Phytohormonal production is frequent in plant-associated bacteria. It ranges from the production of auxins (Spaepen et al., 2007), cytokinins (Arkhipova et al., 2007), gibberellins (Bottini et al., 2004), abscisic acid (Karadeniz et al., 2006), 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Glick et al., 2007) to the synthesis of volatile hydrocarbons (acetoin and 2,3-butanediol) with hormonal activity (Ping and Boland, 2004; Ryu et al., 2003; Kai et al., 2009). Together these compounds function as signaling molecules (Fig. 7) and elicitors of tolerance to abiotic stressors (drought, salinity or nutrient imbalance) in a process termed induced systemic tolerance (IST) (Yang et al., 2009) as well as in triggering the host plant immune system in a process termed induced systemic resistance (ISR) (Ryu et al., 2004). Two well documented examples of these compounds are auxins and ethylene. Microbial production of auxins (indole-3-acetic acid (IAA)) stimulates plant cell proliferation and elongation, resulting in higher total root surface and more efficient water and nutrient uptake (Glick et al., 1998; Patten and Glick, 2002; Spaepen et al., 2008). ACC-deaminase activity lowers the levels of stress ethylene improving plant growth in stress conditions (Glick et al., 1998; Contesto et al., 2008; Tsuchisaka et al., 2009; Bulgarelli et al., 2013).

6.1.3. Biological control and modulation of the host plant immune system

Besides direct plant growth promoting effects, plant-associated microorganisms can have a major impact on the biological control of pathogens and the modulation of the host plant immune system (Fig. 7).

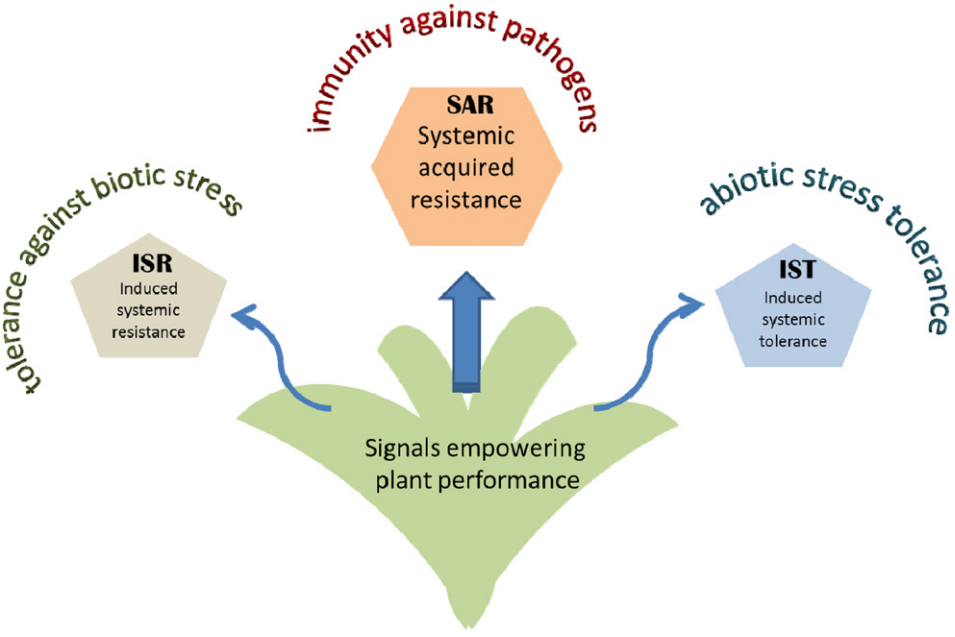


Fig. 7. Role of microbes in empowering plant performance. **ISR** describes a systemic resistance effect triggered by beneficial root-colonizing rhizobacteria in distal not-challenged plant parts of monocotyledons and dicotyledons (De Vleesschauwer et al., 2009; Pieterse et al., 2014). Besides PGPRs, endophytic fungi, and mycorrhizae have been demonstrated to induce resistance against a broad spectrum of pathogens (Balmer et al., 2013). **SAR** represents a systemic induced immune response of plants, contributing to a durable and broad spectrum resistance to a vast majority of harmful microbes, such as bacteria, fungi, or viruses (Vlot et al., 2009). SAR is mainly induced by a local infection of necrotizing pathogens in systemic plant tissue and mobile alarm signals are sent to activate systemic resistance in distal pathogen-free foliage. **IST** is the induced resistance due to abiotic stresses like heat, drought, light or the contact to trace metals (Yang et al., 2009). The border between IST and ISR may be fluent since organic molecules and fungal/microbial elicitors also play a role in both resistance types.

Beneficial microorganisms may prevent pathogen growth and activity via competition for (micro)-nutrients. For example, the production of siderophores may deprive pathogenic bacteria and fungi from iron thereby limiting their pathogenicity (Sharma and Johri, 2003; Compant et al., 2005). Since microorganisms can produce a wide array of compounds with antimicrobial activity (e.g. phenazines) (Berg et al., 2001, Berg, 2009) and hydrolytic enzymes catalyzing cell wall lysis, they will control growth and activity of pathogenic fungi (Krechel et al., 2002). Furthermore, soil-borne microorganisms can also prime or boost the plant's innate immune system in the above-ground plant parts in the process of induced systemic resistance (ISR). Induction of ISR and subsequent signaling cascades results in accelerated responses to pathogen intrusion (Ryu et al., 2004; Van der Ent et al., 2009).

6.1.4. Drought, osmotic stress and freezing resistance

Microorganisms, and especially mycorrhiza, also play crucial roles in plant resistance to drought and osmotic stress and the tolerance against episodes of freezing and thawing. Established mechanisms include the mycelium, which has a smaller diameter than root hairs and therefore better access to bound water (Lehto and Zwiazek, 2011) and various mechanisms protecting the mycorrhizal fungus (and therefore also the plant root) from osmotic stress, such as accumulation of osmolytes (mannitol, trehalose); surface hydrophobicity and bacterial secretion of exopolysaccharides (Evelin et al., 2009; Dimpka et al., 2009).

6.1.5. Impact on soil structure and organic matter content

Plant-associated microbes can influence soil structure. The best known examples are arbuscular mycorrhizal fungi improving soil aggregation through two mechanisms. The first is the production of extraradical mycelium, enmeshing soil particles, physically protecting them from erosion, while the second is the production of amphiphilic molecules, such as glomalin, which promotes the binding of soil particles. Since one gram of grassland can contain as much as 100 m of AMF (arbuscular mycorrhizal fungi) hyphae (Johnson and Gehring, 2007) both mechanisms are relevant at the ecosystem scale. Soil bacteria also produce exopolysaccharides contributing to improved soil structure by stabilizing small aggregates, lining of biopores and mechanical stability (Oades, 1993).

6.1.6. Soil remediation

Finally, plant-associated microorganisms can also play vital roles in the bio-and phytoremediation of contaminated soils and groundwater (Weyens et al., 2009a). Exploring and exploiting the vast metabolic potential of microorganisms (oxidative and peroxidative enzymes in fungi and bacteria, surfactants and alkane dehydrogenases in bacteria) enables more efficient degradation of several complex organic compounds (Taghavi et al., 2005; Barac et al., 2004). For the remediation of soils contaminated with metal(loid)s, the use of plant-associated microorganisms could increase availability, uptake and translocation and decrease phytotoxicity (phytoextraction) and/or contribute to the stabilization of the trace elements in excess (phytostabilization) (Lebeau et al., 2008).

6.2. Diversity versus function: What do we have to know about soil microbes

From all the arguments listed above, it becomes clear that soil microbes contribute to a very significant extent to plant growth on marginal soils. On the other hand, soil amendments that favor microbial activity also have the potential to increase plant growth, through increased mineralization, resistance to plant disease (induced systemic resistance), or drought (induced systemic tolerance) and all other aspects associated with beneficial plant-microbe interaction. As a general rule, we may assume that the more microbes are active, the more they will contribute to soil mineralization processes. Microbes are however sensitive to environmental conditions such as water content, pH or temperature. Hence microbially controlled soil processes are likely to be unstable in a versatile environment, and the loss of a species may lead to the loss of a given soil function. This is where microbial diversity is of importance: the higher it is, the more likely that the loss of a given species (because of a disturbance) is compensated by another one similar in functionality. In this case, this is not the taxonomic diversity per se (Estendorfer et al., 2017) that matters, but rather the functional diversity, defined as the range of processes that a microbial community can contribute to (Heemsbergen, 2004). To measure the contribution of microbial communities in soil processes, both, taxonomic and functional diversity need to be taken into account. High taxonomic diversity could therefore lead to higher stability and resilience of soil processes only if functional redundancy in the community is high. Reversely, some soil processes are dominated by single or a few individual species and therefore the rate of these processes will depend on species identity rather than high functional diversity (Gamfeldt et al., 2008). Hence, a functional trait (such as mineralization and nitrogen fixation) can be a better ecological indicator of soil microbiological quality than the abundance of specific taxa.

7. Indicators and models – Enabling tools for land use planning

Actions to improve the quality and production potential of degraded or low productive soils in Europe should be based on well-defined, objective and justifiable indicators of good soils and soil management, to explain how things are changing over time. The advantage of indicators is that they simplify the quantification of complex phenomena so that the core information can be communicated in a more readily understandable form, even or especially to the public (Bell and Morse, 2008). Nevertheless, no indicator perfectly reflects reality; each has its own limitations. However, when evaluated at regular intervals, indicators will point out the direction of change of current conditions across different units and through time. Environmental indicators to be used at the international level were first introduced by the OECD in 1974, as a "Core Set of Indicators" (OECD, 1974**)** recommended for use by EU Member States. To date, many indicator-based reports are produced by the European Environment Agency, and a set of indicators contributing to the so-called Environmental Sustainability Index (ESI) has been published (World Economic Forum, 2002). This ESI indexes the overall progress towards environmental sustainability in 142 countries (Moldan et al., 2004). In fact, well assigned indicators may become a potent policy instrument to exert peer pressure among regions to perform better.

In addition to taking into account the state and changes in important components of marginal soils, indicators of land use change must particularly reflect human impacts and counter-measures. The DPSIR model - originally developed by the OECD (1993) for environmental indicators, later developed by the EEA (1999) – takes these processes into account and allows comprehensive causal analysis of key factors influencing land use.

Adapted from the original EEA scheme on biodiversity, such a model may include the following levels:

D = Driving Forces: Drivers to show which human activities are causing the relevant burdens to land use.

P = Pressure: Load indicators to express the concrete impact on biological processes involved.

S = State: State indicators describe the state of selected components of the agroecosystem.

I = Impact: Impact indicators highlight changes in biology/chemistry attributed to certain influencing factors.

R = Response: Action indicators measure the extent to which policies and society react to changes in the defined fields of action.

Some of these indicators are purely descriptive, while others focus on performance or efficiency of a process, and finally, in the response section, some can benchmark benefits for the environment or the society.

7.1. Using indicators and models

The first step of indicator building (Cabell and Oelofse, 2012) is to well define the system to be evaluated. In the present case, an assessment is made to determine how well an agricultural ecosystem is meeting the needs and expectations of its present and future users, in order to elaborate methods to sustainably improve soils within marginal and/or degraded lands. In Suppl. Table 2 we have summarized a number of indicators and categorized them according to their environmental, physicochemical and social background.

If the agricultural production system is considered as one compartment in a larger cultured landscape, indicators will have to provide information not only on imbalances, e.g. releases and deficits of the agricultural production system itself, but also on external deposition and off-site effects of emissions resulting from agricultural production, e.g. toxicity of pesticides and their residues towards natural aquatic ecosystems (Hayat et al., 2010).

The amelioration and intensification of productivity on marginal land across Europe encompasses a wide range of biogeophysical and climatic conditions. Naturally, it is relevant to select indicators based on the specific conditions within smaller regions. For this purpose we selected typical soils and farming situations from contrasting regions across Europe, which are described above (see Figs. 2&3), and tailored indicators, measurements and assessment protocols to these situations. A system which is sustainable under given situations may not be resilient to changed boundary conditions or, vice versa, a system that is not resilient today might become resilient if the boundary conditions change. Decision tree analyses may then be used to rule out which scenarios are relevant to investigate.

Both, process-driven dynamic models and conceptual models are useful tools to investigate indicator sensitivity of a system to changed conditions. Notably process-based models have previously been used to evaluate the growth, development and yield of annual and perennial crops under a wide range of conditions (Jones et al., 2003; Keating et al., 2003; Stöckle et al., 2003), including climate change projection across the globe (White et al., 2011; Asseng et al., 2013).

The focus of the conceptual model development is carried out on small selected test site areas described above. An initial step of the conceptual model is based on a decision tree model (Fig. 8) were soil conditions of degraded and marginal soils are identified and evaluated and the corresponding mitigation practice is carried out according to experience that has been obtained from different research studies (Kang et al., 2013; Lasanta et al., 2001; Smith, 2012).

The above decision tree portrays conditions that are often encountered for soils on marginal lands. These soils are poorly developed and have therefore been abandoned due to their low productivity. For each condition there is a suggested mitigation practice, which can also be influenced by other related practices as indicated. For example, it is recommended to vegetate fallow fields. If this does not apply, then erosion is targeted where tillage along slopes and residue retention in the soil would be the recommended mitigation practice. Marginal lands often have nutrient deficiency and are poor in organic material and structure. In this case crop rotation, N-fixing species and amendments are implemented, correspondingly. In case of contamination, it is common to use phytomanagement practices.

7.2. The economic valuation of biodiversity and selected management practices for marginal land

The economic valuation of environmental aspects of land use is a special case of indicator use. It is an essential tool to valuate ecosystem services and productivity of a given site. Confronted with budget constraints farmers need supporting evidence of the benefits of sustainable intensification at the farm level. Without economic valuation of the environment, policy decisions contradicting economic rationality could be supported. In spite of the need for objectively comparable monetary standards, empirical literature investigating the relationship between species diversity and its valuation from a farmer's perspective is still scarce (Finger and Buchmann, 2015). However, it is necessary to understand what intrinsic values like *biodiversity* mean to the general public (Bräuer, 2003; Christie et al., 2006, Feest et al., 2010). Furthermore, the willingness-to-pay (WTP) for species or measures that are unfamiliar or undesired by the general public could yield extremely low values despite the fact that these species could perform indispensable ecological services and thereby contribute indirectly to the farmers' income. Boerema et al. (2016) propose a cascade analysis for the adequate quantification of ecosystem services. The cascade analysis recommends to account for both the ecological and the socio-economic sides for ecosystem service valuation.

Daniels et al. (2017) have proposed an innovative framework effectively integrating ecological and socio-economic aspects into the valuation of biodiversity. Within this wider framework of valuation,

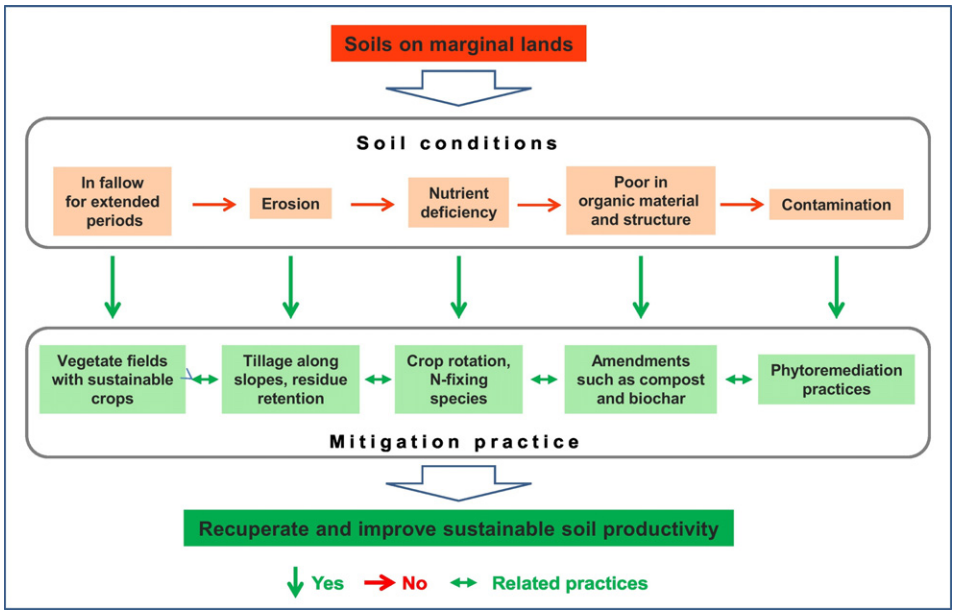


Fig. 8. Decision tree for improving and optimizing the productivity of soils on marginal lands.

functional role-based valuation estimates the indirect value of biodiversity and may hence reveal more objective values than the application of stated preference techniques. The indirect use arises from the functioning of the biological system and if useful to humans, it leads to (bundles of) ecosystem services (Farnsworth et al., 2015).

In a first step the parameters defining the ecosystem properties and parameters related to organisms (e.g. species abundance and composition) in their environment (e.g. plant density, soil properties) have to be selected. The dynamic ecological model will then simulate the interaction between organisms and their environment in multiple scenarios by allowing the ecosystem property parameters (related to organisms and environment) to vary (e.g. less or more biological diversity). The implementation of a production function results in the quantification of ecosystem functioning. In the next step, moving from the ecological to the economic model, a linking function couples the results of ecosystem functioning to the ecosystem services delivered (e.g. nutrient cycling to soil quality regulation). The benefits of enhanced ecosystem services are translated into monetary benefits expressed as net added value, using a direct market approach (Net added value is defined as market price corrected for production costs (€ ton^{-1}, € m^{-3})). This framework allows for the assessment of the indirect value of biodiversity, linking production with a market approach, thereby attributing an objective monetary value to increased species diversity in the provisioning of a marketable good.

7.3. Functional role-based valuation of biodiversity

When dealing with marginal lands, farmers are confronted with constraining ecosystem properties. Solutions/strategies have to be developed based on a combination of management practices, amendments and crop selection, which value (i) the contribution of biodiversity (i.e. microbial diversity) changes to changes in net farm value, and (ii) the contribution of changes in management practices to changes in delivery of ecosystem services. Fig. 9 shows an overview of the approach.

In the first stage of the framework, ecosystem properties are translated to ecosystem functions and changes in services through a production function approach. In a first phase, one generic dynamic simulation model is built for an average site with the use of e.g. the STELLA 10.0.6 model simulating the link between soil biodiversity and its subsequent effects on related ecosystem services: biomass production (food and non-food), soil quality regulation and climate regulation (in Fig. 9, comparison along the X-axis, comparison among colors, where microbial diversity is changed).

In a second phase, the effects of drought and low organic matter on the provisioning of soil services are included, resulting in 2 models (average and marginal lands). Average lands are then compared to untreated marginal lands based on the marginal change in delivering soil services. In Fig. 9 this is shown by comparing within the blue and orange boxes along the Y-axis (dark colors are compared with medium and light colors).

In a third phase, from the models for average and untreated marginal sites, the model is expanded to include the interaction effects of management options (amendments combined with crops) on soil organisms (in Fig. 9, comparison among the green boxes). These options are expected to have a net positive effect on soil organisms as compared to untreated marginal sites, resulting in different provisioning of ecosystem services: (1) differences in changes in soil biodiversity, (2) different potential use of land and biomass during management and (3) new options for potential land use after management. The economic benefit of a management option then depends on the change in delivery of ecosystem services as compared to the situation in an untreated marginal site.

In the second stage of the framework, for each service delivered, changes are valued with an ecological function linked to an economic valuation method. For instance soil fertility such as a decrease/increase in N-fluxes will affect the quantity of fertilizers applied and can be valued using the avoided cost method. The values obtained provide an objective and quantifiable indication of changes in services provided by soil biodiversity and can be considered as an indirect value for the measures applied.

In the final stage of the framework, the (private) costs of the strategies are taken into account and consist of preparation, investment, operational and monitoring costs. Moreover, the potential environmental

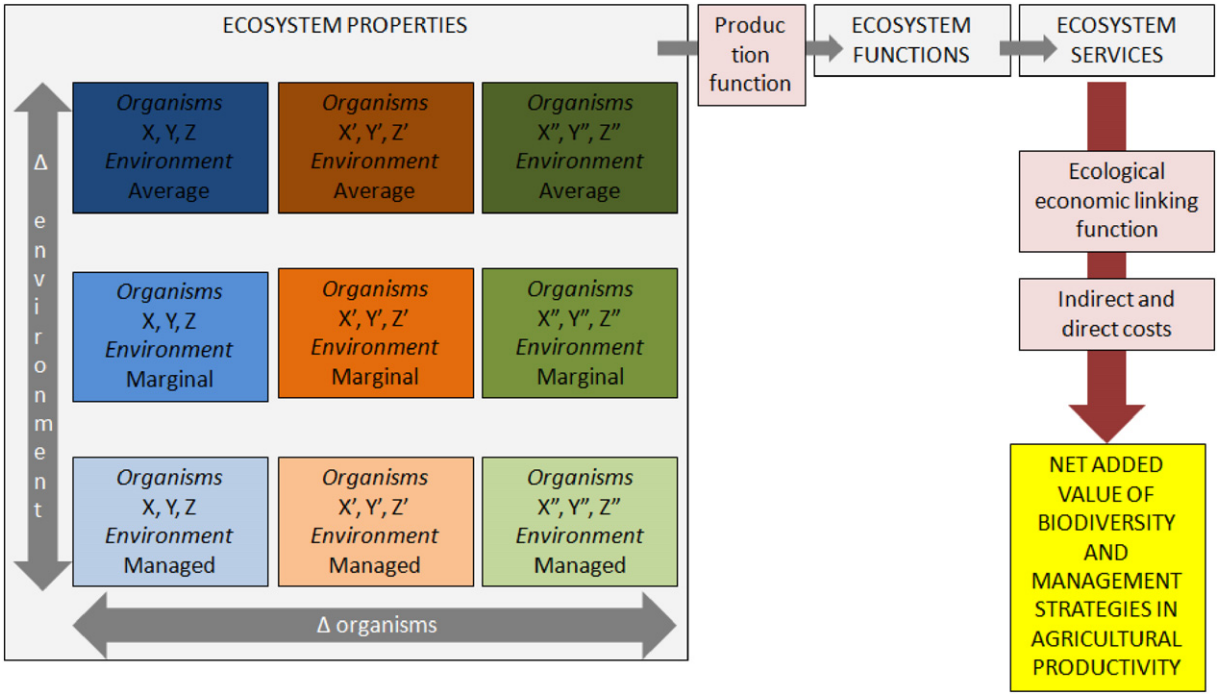


Fig. 9. Interaction effects of management options, amendments combined with crops, on soil organisms. These options are expected to have a net positive effect on soil organisms as compared to untreated marginal sites, resulting in different provisioning of ecosystem services.

impact reduction is included as reduced costs. The effectiveness of strategies in restoring and safeguarding ecosystem services and the role of biodiversity can then be calculated as the net added value of biodiversity and management strategies for agricultural productivity. Model application and validation involves assessing the models accuracy and variability with use of an independent validation dataset. Furthermore, spatial model extrapolation at the regional scale as well as monitoring (over several years) will need to be validated using other extrapolation datasets.

8. Unlock the potential of marginal lands

In our struggle to protect the natural environment and manage the resources of the earth in a sustainable way, soil has been neglected for a long time. Today it is clear that soils are non-renewable resources, at least at human time-scale, under increasing environmental pressure across the world, driven and exacerbated by human activity, such as inappropriate agricultural and forestry practices, urban development, tourism or mining and industrial activities. These activities damage the capacity of soils to continue to perform in its full broad variety of crucial functions and services. Degradation of soils must not be viewed as an isolated problem: it has strong impacts on other areas of common human interest, such as water, human health, climate change, nature and biodiversity protection and food safety. Besides degradation, productivity loss has become a matter of growing concern in our industrialized world. This concern is accentuated by an increasing need for land to meet the demands of the world's ever increasing population. Among the strong drivers of this detrimental situation is the industrialization of food production. We have to outline options for a new form of productivity, in a holistic approach, with emphasis on soil resilience. Otherwise we may soon reach a tipping point where production cannot be made less expensive, without endangering the whole system.

And even more, across the world, valuable agricultural land has become abandoned due to pollution. Such sites remain unproductive in agricultural and ecological context and will not revert to their former state through good agricultural, rangeland management or forestry practice alone. The ecological and human health risk of contaminated soils may be greatest if erosion continues to relocate soil or if the pollutants are resistant to decomposition. Driven by technology feasibility studies of the mid-1980s, the management of contaminated sites has moved from a cost-centered approach in the mid-1970s, to a risk-based approach of the mid-1990s and in the new millennium, where environmental decisions must also fulfill the requirements of sustainable development. With regard to trace element contaminated soils, a variety of physicochemical remediation methods has been adopted, including solidification, electrokinetic soil remediation, encapsulation or soil destructive excavation, followed by washing, pyrolysis or disposal of contaminated soil (Vegter, 2001; Virkutyte et al., 2002, Schwitzguébel et al., 2002). In many cases, these strategies have resulted in criticisms with regards to their high cost, energy intensiveness, site destructiveness, associated logistical problems and growing degree of public dissatisfaction (Yao et al., 2012). The implementation of gentle phytoremediation and rehabilitation strategies using plants and microorganisms to degrade organic contaminants and to stabilize and/or extract plant available heavy metals from contaminated soil, addresses the above mentioned concerns. It is clear that unless the course is reverted, restoration will not occur and the soil will never again be able to complete its full functions.

From an ecological point of view, the rationale for restoration of degraded or marginal land is to recover lost aspects of local biodiversity and ecosystem resilience. From a pragmatic point of view, it is indispensable to recover or repair ecosystems and their capacity to provide a broad array of services and products upon which human economies and human life quality depends. For sure, it is a loss of culture and a loss of patrimony if we decide to abandon agriculture in an area.

And regarding immediate problems, it is of ample importance to counteract extremes in climate caused by ecosystem malfunction. Clear-cut evidence is presented in EU papers that growing crops on degraded land, without trying to revert the degradation status, will not be sustainable, and continued land degradation will be unavoidable if we don't alter the course. Thus, besides scientific progress in understanding soil functioning, it is necessary to mobilize the European Research Area (ERA) to achieve common and well developed strategies to overcome soil degradation problems and to respond to global change issues of high public concern such as restoration of soil life, soil functions and mitigation of soil pollution. Of course this requires sound research and rigorous data analyses in an international context, to provide a data base with highly specific evidence on the one hand, and sufficient broadness on the other to generalize problems and communicate solutions. This is imperative, since many policy makers seem to be unfamiliar with the opportunities for modern, ecologically sound agriculture, or of alternative policies that would enable sustainable farming on marginal and abandoned sites. Decision makers have to recognize that recovery of many other values occurs when smart agriculture is practiced.

Whereas conventional farming uses water soluble, chemical fertilizers, the site-adapted farming applies organic matter in the form of crop residues and other wastes or compost or in the later years also biochar, to enhance biogeochemical nutrient cycling, stimulate soil life and its proliferation effectively (Walmsley and Cerdà, 2017). Invertebrates and microbial activity are pivotal in the fragmentation and decomposition of dead organic material and turn it into humus, and stable substance. The occurrence of microorganisms in the soil depends on many factors e.g. on soil acidity, organic matter, nutrient availability, air and soil humidity, air and soil temperature, soil water, abiotic stressors, etc. Besides providing the human population with food, fodder and agricultural products, the substantial task for the farmer is to take care in returning nutrients extracted from the soil through harvesting.

Scientific progress of the last decades has resulted in a large number of valuable techniques to assess soils, productivity and ecosystem services. However, little of the new science has been shared with farmers, extension services or even with other specialized agricultural scientists and technicians (Scherr and McNeely, 2008). This seems especially true for applied sciences, dealing with real-life innovations that local people can make to modify ecological impacts of management activities. Agricultural advisory services, even if public or on academic extension services, rarely address landscape management issues (Scherr and McNeely, 2008). But it is now necessary to translate exactly these insights into tools for farmers and stakeholders for site specific assessment and treatment of field sites and knowledge-based practical instructions, on a regional scale. This requires that local stakeholders are informed about the problem, are correctly consulted, and that they get the best available tools at hand to take action, ideally assisted by scientific guidance (REVIT project, 2007).

Thus, applied research for a sustainable and ecologically compatible land use aiming at sufficient food production is ever so important and needs to be disseminated to stakeholders (Schröder et al., 2002, 2003, 2008b). Precise farming techniques will be helpful to re-establish soil life as first priority, and to re-introduce cycling of nutrients. Eco-agriculture approaches will be needed to repair lost functions, and to conserve wildlife (Scherr and McNeely, 2008). Decision support systems considering energy efficiency, variations in climate conditions, cropping systems and production goals between regions will implement regional welfare.

9. Conclusions

To embrace these goals in marginal land, agricultural and conservation innovators have to pursue strategies to minimize agricultural pollution of natural habitats, manage conventional cropping systems in ways that enhance habitat quality, and design farming systems to mimic the structure and function of natural ecosystems. A reliable strategy is

needed to combine and communicate the available tools so that agricultural output is maintained or even increased, production costs stay stable and the market value of the products increases.

The challenge is no longer simply to maximize productivity of a single crop, but to optimize farming across a far more complex landscape of production, environmental, and social outcomes. When agriculture thrives under the auspices of land-owners educated in sustainable land use, the potential of marginal lands will be unlocked and strengthened, and local stakeholders will defend their region from further degradation to establish economically sound management systems.

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Appendix A. Supplementary data

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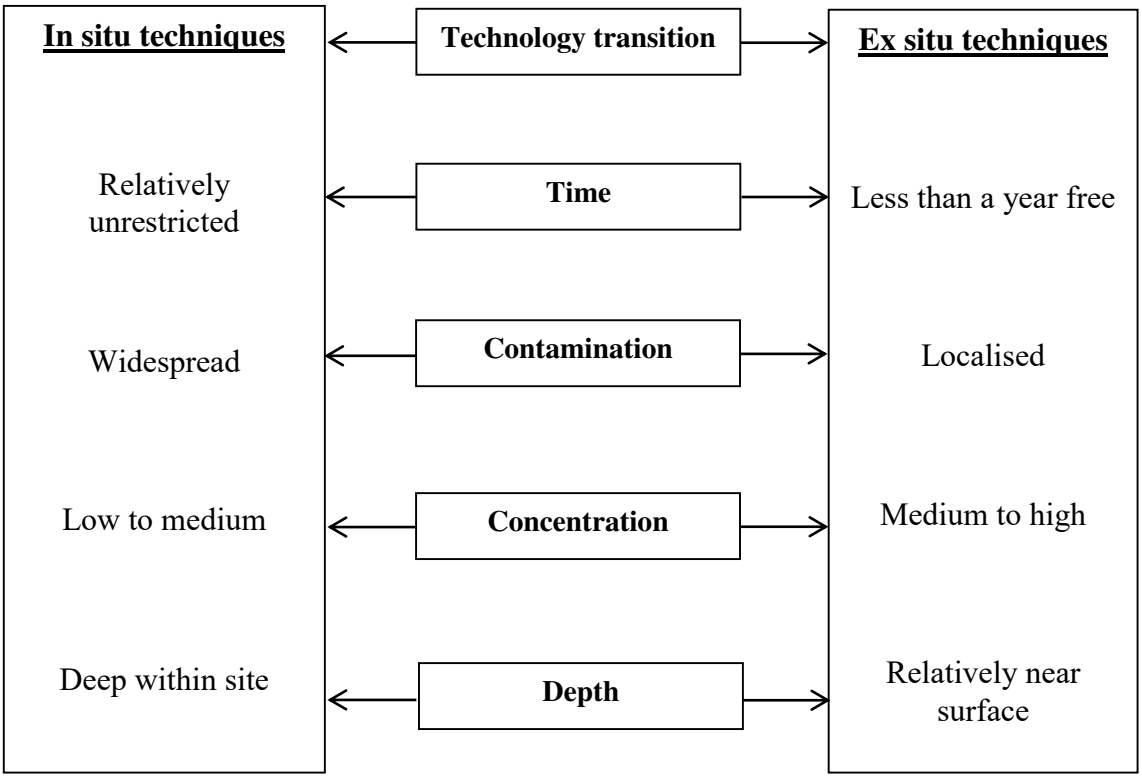
Appendix M3 (Supplements III)

Figures

Figure S1 Factors involved in the choice of the remediation technology



Tables

Table S1 Composting conditions required to ensure waste hygienization.

According to US Environmental Protection Agency regulations (EPA, 2002), maintaining a minimum temperature of the composting mass of 55° C for 3 days (aerated static pile or in-vessel) or 15 days with 5 turns is recommended to meet the regulatory requirements of class A fertilizers, and a minimum of 40° C for 5 days - during which temperature should exceed 55° C for at least 4 h - to meet class B fertilizer requirements.

Method of composting	Temperature [°C]	Turn number of composting mass	Holding time (weeks)
Composting in pile	55	2	5
Composting in pile	65-70	1	2
Composting in reactors	60	1	-

Additional information to Table S1 (Essential factors)
Among factors influencing the composting process, the more important are (a) the **C/N ratio** - this relationship depends on the dynamics of microbial processes. The optimal ratio is 25-30. High C/N ratios make this process very slow as there is an excess of degradable substrate for the microorganisms. In the low C/N ratios microbiological processes stop, which may lead to leaching nitrogen from composting mass; (b) **pH**: optimum values are between 5.5 and 8.0. Usually pH is not important for composting. However, it becomes relevant in controlling N-losses by ammonia volatilization, especially at high pH, e.g. >7.5; (c) **aeration** is an important factor for composting, with the optimum O_2 concentration between 15% and 20%. Turning the compost pile provides air circulation, temperature maintenance and proper development of aerobic microorganisms which determine the speed of the composting process; and (d) **moisture**: The optimum water content for composting varies between 50–60%. Moisture contents higher than 60% inhibit the composting process due to low oxygen concentration, on the other hand, if moisture is too low, the composting process will be hampered.

Table S2 Examples of field trials to demonstrate the phytomanagement of trace element contaminated soils

Plant species	Trace element	Remediation type	Reference
Berkheya coddii	Ni, Co	Phytoextraction	Keeling et al., 2003
Alyssum murale			Bani et al., 2017
Geissois pruinosa Brongn. & Gris	Ni	Hyperaccumulators Phytoextraction	Losfeld et al., 2015
Psychotria douarrei	Ni		Grison et al., 2013
Grevillea exul Lindl.	Mn	Hyperaccumulator Phytoextraction	Losfeld et al., 2015, Escande et al., 2015
Betula celtiberica	As	Bioaugmented Phytoextraction	Mesa et al., 2017
Pityrogramma calomelanos var. Austroamericana, Pteris vittata	As	Hyperaccumulators, Phytoextraction	Niazi et al., 2012
Rosa multiflora Thunb. ex Murray, *Sida hermaphrodita* Rusby	Cr, Ni, Cu, Zn, and Cd	Phytoextraction	Antonkiewicz et al., 2017
Iberis intermedia	Tl	Hyperaccumulator Phytoextraction	Grison et al., 2015b
Anthyllis vulneraria subsp. *carpatica*	Zn	Hyperaccumulator Phytoextraction	Frérot et al., 2006; Mahieu et al., 2013; Grison, 2015; Grison et al., 2015a
Noccaea caerulescens	Zn	Hyperaccumulator Phytoextraction	Losfeld et al., 2012; Grison, 2015
Noccaea caerulescens, Noccaea praecox, Arabidopsis halleri	Zn, Cd, Pb	Hyperaccumulators Phytoextraction	Tlustoš et al., 2016
Sedum plumbizincicola	Zn, Cd	Hyperaccumulator intercropping with maize, phytoextraction	Deng et al., 2016
Sedum plumbizincicola intercropped with *Medicago sativa*	PCB, Cd, Cu	Hyperaccumulator intercropping with alfalfa, phytoextraction, rhizodegradation	Wu et al., 2012
Sedum alfredii co-planted with *Alocasia marorrhiza*	Metals, PAH	Hyperaccumulator co-planting with elephant ear, phytoextraction, rhizodegradation	Qiu et al., 2014
poplar cultivar 'SKADO' (*Populus maximowiczii x P. trichocarpa*)	Zn, Cd	Phytoextraction	Bert et al., 2017
Salix 'Tora'	Cd, Zn	Phytoextraction	Delplanque et al., 2013
Salix viminalis and *Salix alba x alba* clones	Zn, Cd	Phytoextraction	Janssen et al., 2015
Willows, poplars	Zn, Cd	Phytoextraction	Kidd et al., 2015
Willows, poplars	Zn, Cd, Pb	Phytoextraction	Kacalkova et al., 2015; Kubatova et al., 2016
Salix schwerinii x Salix viminalis x S. viminalis, *Salix x smithiana* clone S-218, *Populus maximowiczii* x *Populus nigra*	Zn, Cd, Pb	Phytoextraction	Zarubova et al., 2015
Populus deltoides Dvina, Populus x canadensis Orion, Pteris vittata (Chinese brake fern)	As, Al, Fe, Cu, Co, Cr, Pb	co-planting system phytoextraction	Ciurli et al., 2014
Industrial hemp and white lupin rotation	Zn, Cd, Ni, Cu	Winter crop/summer crop, phytoextraction	Fumagalli et al., 2014
Rapeseed and rice rotation	Cd		Yu Lingling et al., 2014
Sunflower		Secondary metal accumulator, phytoextraction	Kolbas et al., 2011
Sunflower		Secondary metal accumulator, phytoextraction	Kidd et al., 2015
maize (*Zea mays* L.), sunflower (*Helianthus annuus L.*), willow (*Salix x smithiana* Willd.), and poplar (*Popuus nigra* L. x *P. maximowiezii*)	Ni, Pb, Cd, Cr	phytoextraction	Kacalkova et al., 2014
Maize, Vetiver	Pb	Phytoextraction aided by citric acid	Freitas et al., 2013
Pelargonium	Pb, Cd, Zn, Cu, As	Phytoextraction	Shahid et al., 2012
Nicotiana tabacum	Zn, Cd, Cu	Secondary metal accumulators, phytoextraction	Kidd et al., 2015; Gonsalvesh et al., 2016
Brassica juncea	Cd	phytoextraction	Parisien et al., 2015
Festuca arvernensis	Zn, Cd	Phytostabilisation	Frérot et al., 2006

Miscanthus	Zn, Pb, Cd, etc.	Phytostabilisation	Nsanganwimana et al., 2014*a*, 2015, 2016; Kidd et al., 2015
Arundo donax	Zn, Pb, Cd, etc.	Phytostabilisation	Nsanganwimana et al., 2014*b*
Holcus lanatus L.	Cd, Pb, Zn, As		Friesl-Hanl et al., 2009
Salix spp., *Populus nigra* L., *Agrostis capillaris* L.	Cu	(aided) phytostabilisation	Touceda-Gonzales et al., 2017*a*
Andropogon schirensis, Eragrostis racemosa, Loudetia simplex	Cu	P hytostabilisation	Boisson et al., 2016

Additional information to Table S2 Phytomanagement experiments at field scale established before 2015, including phytoextraction, phytostabilisation and in situ stabilization/phytoexclusion options, were reviewed by Vangronsveld et al., (2009), Mench et al., (2010) and Kidd et al., (2015). Precautions for use are needed with invasive plant species (Che-Castaldo and Inouye, 2015; Losfeld et al., 2015). White lupin as winter crop in sequence with a metal-accumulator summer crop such as industrial hemp can improve the recovery of soil quality during the phytoextraction period, i.e. green manure avoiding the application of chemical amendments, increase in soil bacteria and metal bioavailability (Fumagalli et al., 2014). Planting oilseed rape increased the uptake of Cd by the successive rice crop compared with a previous fallow treatment (Yu Lingling et al., 2014). The beneficial effect of compost incorporation into Cu-contaminated soils, alone and in combination with other amendments, and crop rotation is generally marked on both plant biomass and microbial communities (Kolbas et al., 2011; Touceda-Gonzales et al., 2017*a*). Hyperaccumulators are currently developed for Ni phytomining and producing biosourced Cu-, Mn, and Co-ecocatalysts for the green chemistry (Losfeld et al., 2015). Biocatalysis, based on use of metal(loid) species originating from plant biomasses with high metal(loid) concentrations (unusual oxidation levels, new associated chemical species, and effects of synergy) is an emerging technique (Grison et al., 2015*a*). Intercropping with *Fabaceae* is relevant in case of mixed soil contamination. For example, liming with *Sedum plumbizincicola* intercropped with *Medicago sativa* enhanced soil PCB degradation by 25% and removal rates of Cd and Cu (Wu et al., 2012). Co-planting system, i.e. As-hyperaccumulator fern *Pteris vittata* and poplar clones, was suitable for phytoextracting As and metals at a contaminated dumping site, albeit the efficiency depended on irrigation, soil tillage and soil amendments for enhancing plant growth (Ciurli et al., 2014). Co-planting *S. alfredii* and *Alocasia marorrhiza* decreased the DTPA-extractable Zn, Cd, and Cu and benzo[a]pyrene in a sludge as compared with the unplanted sludge (Qiu et al., 2014). In a pot experiment, remediation of a spiked Cd and PAH co-contaminated soil by *S. alfredii* was enhanced by application of pig manure vermicompost (Wang et al., 2012).

Table S3 Selected indicators and measurements to assist smart intensification of crop production. The table also indicates clearly that intensification will have an influence on social and socioeconomic indicators. Both, driving forces and responses from stakeholders, policy makers and the local population are strong. The trigger types include driving forces (D), pressure (P), state (S), impact (I) and response (R). More detailed information is given in M3.

Indicator	Trigger type D,P,S,I,R	Related soil measurements	Related meteorological measurements	Other related assessments
Ecological indicators				
Carbon cycling	D, R	Soil org matter, microbial biomass, microbial community composition	Soil temperature, precipitation	
Soil fertility	S	Nutrient analyses, microbial activity, organic matter, rooting depth, pH etc	Soil temperature, precipitation	
Soil resilience	S	Soil erosion, soil compaction, organic carbon, pH, N and other nutrients,	Soil temperature, precipitation	Nutrient contents, water retention
Biodiversity	P, R	Plant species, soil fauna, microbial biomass, microbial community composition; fungal/bacterial community biomass ratio,		
Biogeophysical indicators				
Carbon footprint	D, R	Soil GHG emissions, C/N-ratio, DOM	Temp., precipitation, solar radiation	
Water quality	S	Concentration of N, P, pesticides etc in water	Rainfall frequency, surface runoff	
Soil physical properties	S	Bulk density, texture, electrical conductivity, infiltration rate, soil aggregates	Soil temp., conductivity	Infiltration, oxygen levels, texture composition
Economic indicators				
Yield quantity and quality	D, I	Crop yield, nutritive value and contamination	Temp., precipitation, solar radiation	
Profitability	D, R			annual income from farm
Land value / ownership	D, R			farm real estate data, rent data
Health of population	D			
Economic growth	D, R			market price of harvests
Social indicators				
Status of infrastructure	D, R			public/private transport
Sustainable human activity	D, R			
Job opportunities	D, I, R			unemployment rate
Local interest (locally important specialty crops)	D, I, R			tourism?

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