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The influence of different land use intensities on plant-associated bacterial communities of *Dactylis glomerata* L.

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Für meine Mutter,

eine der stärksten und inspirierendsten Personen, die ich kenne,

und meinen Vater,

einer der geduldigsten Menschen und weil er immer für mich da ist.

„Wege entstehen dadurch, dass man sie geht“

Franz Kafka

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Zusammenfassung

Die Intensivierung der Landnutzung ist die wichtigste anthropogene Ursache für den Verlust der biologischen Vielfalt in semi-natürlichen Grünlandhabitaten in Europa und stellt eine große Bedrohung für die Gesundheit und das Funktionieren der Ökosystemleistungen dar. Das größte Reservoir für die biologische Vielfalt ist das Habitat des Bodens, welches hauptsächlich aus Mikroorganismen wie Bakterien besteht. Sie spielen eine entscheidende Rolle im globalen Nährstoffkreislauf und bei der Zersetzung organischer Stoffe. Darüber hinaus beherbergen die bakteriellen Gemeinschaften im Boden viele pflanzenassoziierte Bakterien, die die Rhizosphäre und das Wurzelinnere der Pflanzen besiedeln. Pflanzenassoziierte Bakterien spielen eine maßgebliche Rolle für die Fitness, Entwicklung, Ernährung und Widerstandsfähigkeit von Pflanzen gegenüber Umweltstressoren und bilden zusammen mit ihrem Wirt eine funktionelle Einheit, die als Pflanzenholobiont bezeichnet wird. Trotz ihrer Bedeutung für die Gesundheit der Pflanze ist die Auswirkung von Intensivierung der Landnutzung auf pflanzenassoziierte Bakterien unklar. Um zu verstehen, wie sich die Landnutzungsintensität (LUI) auf die komplexe Beziehung zwischen der Pflanze und den mit ihr assoziierten Bakteriengemeinschaften auswirkt ist es wichtig, Einblicke in die Faktoren zu gewinnen, die die Vielfalt und Zusammensetzung der assoziierten Bakteriengemeinschaften beeinflussen.

In diesem Zusammenhang zielt die aktuelle Studie darauf ab, die Vielfalt und Zusammensetzung der Bakterien im Boden, der Rhizosphäre und der Wurzelendosphäre des mehrjährigen Futtergrases *Dactylis glomerata* L. zu analysieren und deren Reaktion auf LUI zu beobachten. Die über verschiedenen Jahreszeiten verteilten Probenahmen ermöglichten die Untersuchung des Einflusses von LUI während verschiedener Entwicklungsstadien der Pflanze (vegetatives, reproduktives und Seneszenz Stadium), die Beschreibung von LUI-abhängigen Kernmikrobiomen und die anschließende Identifizierung potenzieller bakterieller Schlüsselakteure innerhalb des Pflanzenholobionts.

Die Analyse zeigte, dass sich sowohl die Vielfalt als auch die Zusammensetzung der bakteriellen Gemeinschaft in der Wurzelendosphäre erheblich von den anderen Kompartimenten unterscheidet, was zu einer sehr unterschiedlichen bakteriellen Struktur führt. Im Gegensatz dazu waren die bakterielle Vielfalt und Zusammensetzung in der Rhizosphäre und im Boden vergleichbar. Die LUI hatte während aller Probenahmezeiten einen starken Einfluss auf die bakteriellen Gemeinschaften im Boden und beeinflusste sowohl das Vorhandensein oder Fehlen als auch die Häufigkeit der bakteriellen Taxa. Das Ausmaß der Auswirkungen von LUI nahm jedoch in der Rhizosphäre ab, in der lediglich die An- und Abwesenheit bestimmter Bakterien festgestellt werden konnte. Trotz des Einflusses von LUI auf den Boden und die Rhizosphäre blieben die bakteriellen Zusammensetzungen in der Wurzelendosphäre relativ stabil

und kongruent. Nur einige wenige, seltene Taxa waren in den verschiedenen Entwicklungsstadien der Pflanzen betroffen. Darüber hinaus wurde ein signifikanter Wechsel in der An- und Abwesenheit bestimmter Taxa während der Reproduktionsphase der Pflanzen beobachtet. Diese Veränderung in der Zusammensetzung der Bakterien konnte auf eine veränderte Nährstoffverfügbarkeit zurückgeführt werden, die sich in der Menge an Nitrat und Ammonium im Boden widerspiegelt und stark mit der LUI korreliert. Somit wurde die LUI als ein Hauptfaktor für Veränderungen identifiziert, da eine Feedbackschleife ausgelöst wird. Dieser Effekt ist vor allem auf Flächen mit niedriger LUI zu beobachten. Die geringe Nährstoffverfügbarkeit übt Druck auf die Selektionskraft der Pflanze aus, um eine vorteilhafte Kolonisierung von endophytischen Gemeinschaften zu etablieren. Dadurch kompensiert die Pflanze ihre wechselnden Stoffwechselbedürfnisse in verschiedenen Entwicklungsstadien. Infolgedessen konnte bei niedriger LUI eine verstärkte Interaktion zwischen der Pflanze und dem sie umgebenden Boden festgestellt werden. Diese Ergebnisse wurden durch eine Analyse der Kernmikrobiome zwischen den verschiedenen LUI-Niveaus ergänzt, welche eine höhere Variabilität bei niedriger LUI über die Entwicklungsstadien der Pflanze sowohl in der Rhizosphäre als auch in der Endosphäre ergab. Trotz dieser dynamischen Anpassung wurde eine Reihe von bakteriellen Taxa unabhängig von den Entwicklungsstadien der Pflanze und der LUI gefunden, die so genannten potenziellen Schlüsseltaxa, die vermutlich eine zentrale Funktion innerhalb des Pflanzenholobionten haben. Dazu gehörten die Gattungen *Pseudomonas, Rhizobium* und *Bradyrhizobium* in der Wurzelendosphäre und *Rhodoplanes*, *Methylibium*, *Kaistobacter* und *Bradyrhizobium* in der Rhizosphäre. Diese Ergebnisse liefern erste Einblicke in die Identifizierung einer "gesunden" pflanzenassoziierten Bakteriengemeinschaft.

Insgesamt tragen die Ergebnisse dieser Arbeit wesentlich zum Grundwissen der Bildung von pflanzenassoziierten Bakteriengemeinschaften als Reaktion auf LUI in realen Landnutzungsszenarien bei. Die Ergebnisse könnten durch die Analyse von Netzwerken des gemeinsamen Vorkommens von Schlüsseltaxa mit anschließender Funktionsanalyse ergänzt werden. Diese Erkenntnisse würden unser Verständnis der komplexen Wechselwirkungen zwischen Pflanzen und ihren assoziierten Mikrobiomen sowie der Auswirkungen der Landnutzungsintensität auf die biologische Vielfalt und die Ökosystemprozesse erheblich verbessern. In der Folge ist die Entwicklung eines nachhaltigen Grünlandmanagementkonzepts möglich, das den weiteren Verlust der biologischen Diversität einzudämmen helfen könnte.

Summary

Land use intensification is the most important anthropogenic cause of biodiversity loss in semi-natural grasslands in Europe and poses a major threat to the health and functioning of ecosystem services. The largest reservoir for biodiversity is the soil habitat, which primarily consists of microorganisms such as bacteria. They play a key role in global nutrient cycling and organic matter decomposition. In addition, soil bacterial communities harbor many putative plant-associated bacteria that colonize the rhizosphere and root interior of plants. Plant-associated bacteria play a critical role in plant fitness, development, nutrition, and resistance to environmental stressors, and together with their host form a functional unit called the plant holobiont. However, despite the underlying significance, the response of plant-associated bacteria towards land use intensification remains unclear. To achieve a profound understanding on how land use intensity (LUI) affects the complex relationship between the plant and its associated bacterial communities, it is essential to gain insights into the drivers that influence the diversity and composition of bacterial assemblages associated with the plant holobiont.

In this context, the current study aimed at observing the diversity and composition of bacteria associated with the bulk soil, rhizosphere and root endosphere of the perennial forage grass *Dactylis glomerata* L. in response to LUI. Different sampling seasons allowed the investigation of the influence of LUI during different plant developmental stages (vegetative, reproductive, and senescence stage), the description of LUI-dependent core microbiomes and the subsequent identification of potential key bacterial players within the plant holobiont.

The analysis showed that both the diversity and composition of the bacterial community in the root endosphere differed considerably from the other compartments, resulting in a very distinct community pattern. In contrast, bacterial diversity and composition in the rhizosphere and soil were comparable. LUI had a strong impact on soil bacterial communities during all sampling seasons, affecting not only the presence or absence, but also the abundance of bacterial taxa. The degree of the impact of LUI, however, decreased in the rhizosphere, where only the presence and absence of certain bacteria was noted. In the root endosphere, however, bacterial assemblages remained relatively stable and congruent despite the influence of LUI on the soil and rhizosphere. Only a few low abundant species were affected throughout the different plant developmental stages. In addition, a significant change in the presence and absence of certain taxa during the plants reproductive stage was observed. This shift in community composition could be attributed to a change in nutrient availability as reflected by the amount of nitrate and ammonium in the bulk soil, which strongly correlated with LUI levels. Hence, LUI was identified as a major driver of changes by triggering a feedback loop. This effect is most evident on low LUI sites. The low nutrient availability imposes pressure on the plant's selective force to estab-

lish beneficial colonization of endophytic communities that compensate for the changing metabolic demands of the different developmental stages. As a result, the interaction between the plant and its surrounding soil was increased on low LUIs. These results were complemented by analysis of the core microbiomes between the different LUI levels, which showed higher variability at low LUI throughout the developmental stages of the plant in both the rhizosphere and endosphere. Despite this dynamic adaption, a set of bacterial taxa was persistently found irrespective of plant developmental stages and LUI, the so-called putative keystone taxa, which supposedly have important function within the plant holobiont. These included the genera *Pseudomonas, Rhizobium, and Bradyrhizobium* in the root endosphere and *Rhodoplanes, Methylibium, Kaistobacter,* and *Bradyrhizobium* in the rhizosphere. These results provide first insights into the identification of a “healthy” plant-associated bacterial community.

Overall, the results of this work contributed significantly to the understanding of the formation of plant-associated bacterial assemblages in response to LUI in real management scenarios. The results could be complemented by analysis of co-occurrence networks of key taxa followed by functional analysis. These insights would greatly benefit our understanding of the complex interactions between plants and their associated microbiomes, as well as the effects of land use intensity on biodiversity and ecosystem processes, so that a sustainable grassland management approach can be developed to mitigate further biodiversity loss.

1 Introduction

1.1 Soil biodiversity

1.1.4 The role of biodiversity on ecosystem services

Biodiversity is referred to as the web of life (Morton, Steve & Hill, 2006). Soil biodiversity represents one of the main global reservoirs of biodiversity with more than 40% of living organisms directly associated with soil throughout their life cycle, exceeding the biodiversity of other terrestrial systems by orders of magnitude (Bardgett & van der Putten, 2014; Decaëns et al., 2006; FAO, 2020), which can be attributed to the exceptional heterogeneity of soils (Sikorski, 2015). There are more individual organisms in a teaspoon full of soil than people on this planet (Schoonover & Crim, 2015). The term biodiversity however, does not only refer to the variety of living organisms and the number of species, but also includes the functional variety of traits, which determine the ecological function within an ecosystem, evenness of species distribution (local and global), the genetic diversity, or genetic material within each species as well as their ecological and evolutionary processes that contribute to their functionality and adaptability to an ecosystem (Cardinale et al., 2012; Morton et al., 2014). An ecosystem is not only the physical environment, but it consists of intricate webs of interactions, not only between one or more trophic levels of organisms, but also their non-living environments, which act as a functional unit. The multitude of organisms produce energy and biomass, consume water and nutrients, and thereby alter the biological, chemical and physical properties of their surrounding environments (Morton et al., 2014; FAO, 2020). These processes are a result of living systems that contribute to the maintenance and replenishment of ecosystems and consequently benefit their members by producing a variety of goods and services, the so-called ecosystem services (Millennium Ecosystem Assessment, 2005). Global organisations like the Food and Agriculture Organization of the United Nations (FAO) recognize ecosystem services as the engine of the environment that are essential to life. The activity of soil organisms is crucial for soil functioning as it supports fundamental processes and provides key ecosystem services, directly as well as indirectly (FAO, 2020). The FAO delineates four functional classifications of ecosystem services, which are comprised of the provisioning, regulating, cultural, and supporting services (Teeb, 2011).

Provisioning services are described as any type of material or energy output for humans that can be obtained from an ecosystem. These services are food products derived from plants and animals, raw materials, medicinal resources, clean water and other goods, which do not only provide economic value but can also directly affect livelihoods and heavily depend on soil functioning (Costanza et al., 1997; Kibblewhite et al., 2008).

Regulating services are defined as essential for the perpetuation of ecosystems through the maintenance of climate, quality of air, carbon sequestration and storage, waste-water treatment, prevention of erosion, maintenance of soil fertility, disease control, and crop pollination (Pulleman et al., 2012). Even though the value of regulating services has not been directly captured in terms of its economic value as it is not sold on markets, it is indispensable for human well-being (Balasubramanian, 2019). For instance, soil organisms drive cycling of energy and nutrients, thereby enhancing soil fertility and agricultural production. Regulation is based on activities of living organisms and damage can result in substantial losses that are difficult to restore (El Mujtar et al., 2019; Pulleman et al., 2012).

Cultural services are summarized as non-material benefits obtained from ecosystems through aesthetic, spiritual, and psychological value (Teeb, 2011). These include recreation and the restoration of physical and mental health or opportunities for tourism, which is also of significant economic importance for many countries. Soils support a variety of vegetation throughout different landscapes. The value of cultural services is among the highest values associated with nature for human health and well-being (Costanza et al., 2014; FAO, 2020). Furthermore, the provisioning and regulating services are strongly interrelated with cultural services (Chan et al., 2012).

The supporting services are described as fundamental for the production of all other ecosystems and their services through providing habitats for living organisms and maintaining the biological diversity of these. Soil structure formation or cycling of nutrients within each ecosystem can provide different living spaces, which can be crucial for species' lifestyle (El Mujtar et al., 2019). Therefore, supporting services also maintain genetic diversity (i.e. "the variety of genes between, and within, species populations") as well as the diversity of complex processes that contribute to the other ecosystem services.

So-called "biodiversity hotspots" like the soil habitat are areas with exceptionally high numbers of species and a higher genetic diversity compared to other habitats and contribute to the ecosystem services mentioned above to a large extent (Millennium Ecosystem Assessment, 2005; Teeb, 2011).

1.1.5 Major threat - biodiversity is declining

With almost 8 billion people on the planet, ecosystems experience a decline (Morton et al., 2014). Human activities have been and are continuing to change the environment on all scales, from local to global range (Hooper et al., 2005). As the human population has grown and technology has become more abundant and influential, the scope and nature of this modification has significantly changed (Millennium Ecosystem Assessment, 2005). As a result, ecosystems

rapidly lose functional, taxonomic, phylogenetic, and genetic diversity, leading to a decline of ecosystem services, landscape and climate changes (Hooper et al., 2005; Naeem et al., 2012). Several studies suggested that planet Earth already has entered the sixth mass extinction event and moreover, that human activities accelerated the rate of species' extinctions (Barnosky et al., 2011; Naeem et al., 2012). The nature of this significant human impact has led to the development of a term called the "Anthropocene", which has been proposed as a new geological era, an epoch that is dominated by human activity (Cooper et al., 2018; Steffen et al., 2007). Hence, it is more topical than ever as the loss of soil biodiversity and ecosystem services lead to a major threat for human well-being (Cardinale et al., 2012; El Mujtar et al., 2019). As we are facing the challenge of the growing demand for goods and services, while at the same time needing to counteract negative perturbations for a sustainable ecosystem-service delivery, it is of great importance to understand how human activities affect soil biodiversity (Allan et al., 2015). A major requirement to prevent biodiversity loss from a value chain perspective is the development of predictive models as well as recommendations for political decision-makers using scientifically robust metrics and indicators (Crenna et al., 2020). Thus, it is crucial to understand the underlying ecological processes that concatenate soil biodiversity and ecosystem functioning (Gonzalez et al., 2020). Loss of soil biodiversity has been demonstrated to fundamentally change ecological processes that control fluxes of energy, cycling of nutrients, and organic matter composition within an environment (Beaumelle et al., 2020; Hooper et al., 2012) and can deplete the efficiency of an ecosystem to gain resource, produce biomass, and recycle nutrients (Morton et al., 2014; Weisser et al., 2017). In their report "Making Peace with Nature" (United Nations Environment Programme, 2021), the "United Nations Environment Programme" (UNEP) has communicated the global impact of biodiversity loss, with land use intensification representing the forefront of human activities that drive global and regional loss of biodiversity in terms of reducing plant, animal, and microbial biodiversity (Allan et al., 2015; Barros-Rodríguez et al., 2021; Sala et al., 2000; United Nations Environment Programme, 2021).

1.1.6 The showcase of grassland ecosystems

Grasslands, defined as land that is dominated by grasses with less than 10 percent trees or shrubs, are the major ecosystems of the world, covering about one-third of the total land area (Bengtsson et al., 2019). They occur across various regions, from tropical to temperate areas, and based on their ecology can be categorized as meadows, prairies, rangelands, savannas, steppes, tundras, veldts, and pastures (Gibson, 2009). The ecological basis of these land types is mainly determined by climate factors, such as temperature and soil moisture (Sanderson et al., 2015). In general, there are three major types of agricultural grassland systems: natural,

semi-natural, and improved grasslands (Bengtsson et al., 2019). Natural grasslands like savannas, prairies, steppes, tundras, pampas, or veldts are characterized by natural disturbances related to climate, fire, wildlife, or the potential for livestock grazing (Bengtsson et al., 2019; Gibson, 2009). Semi-natural grasslands like meadows or pastures are a result of human activity, mainly altered by and for agriculture. They are grazed by livestock or maintained through cutting for hay or silage. Improved grasslands on the other hand are created through the human introduction of agricultural plants, high stocking rates, and fertilization (Bengtsson et al., 2019).

In Europe, semi-natural grasslands are one of the most valuable land use forms (Isselstein, 2005). Moreover, grassland ecosystems provide a great amount of ecosystem services. The European Commission reported that permanent grassland accounted for 31.2% of the utilized agricultural area (Eurostat, 2020). The EU livestock industry consumes around 500 million tonnes of feed annually. About 40% of this amount is grass [expressed in dry matter] (Lesschen et al., 2011). Besides the production of goods (i.e. provisioning services), grasslands provide other important biodiversity-based functions and services. These include regulating ecosystem services like carbon sequestration, pollination, regulation of erosion, water run-off, improvement of water quality, and cultural ecosystem services through providing a source of aesthetic influence and recreation as well as supporting services like the formation of soil structure, nutrient cycling, and conservation of biodiversity (Allan et al., 2015; Carlier et al., 2009; Hopkins & Holz, 2006; Isselstein, 2005; O'Mara, 2012). The value of services provided by grasslands can be correlated to biodiversity and strongly depends on the goal of stakeholder communities. Allan et al. (2015) showed that on one hand a loss of biodiversity had negative effects on cultural services, but on the other hand had weak or even positive effects on provisioning services and thus led to a trade-off between biodiversity and ecosystem functions and services. Zavaleta and Hulvey (2004) found that loss in diversity can lead to the reduction of grassland resistance against biological invaders, which can further affect other ecosystem processes. Other experiments and observations indicate that the diversity of different groups of organisms has varying effects on functions, with multiple trophic groups involved in providing a particular function like the diversity of composers, predators, and plants determining primary productivity (Felipe-Lucia et al., 2020). Thus, loss in diversity is likely to substantially impair ecosystem functions and services, subsequently impacting human well-being (Newbold et al., 2015).

Soil biodiversity in particular is threatened by intensification of agriculture, which is negatively impacting the delivery of ecosystem goods and services (Tsiafouli et al., 2015). As grasslands are considered biodiversity hotspots and the most diverse ecosystem (Le Provost et al., 2021; Regan et al., 2014; Simons et al., 2017), they are a key ecosystem for biodiversity conservation through its high species richness at a small spatial scale (Raatikainen et al., 2009; Wilson et al., 2012). Hence, they are of major importance for biodiversity research and the understanding

on how to sustain soil functions under the growing pressure of climate change. Global assessments have already shown that species richness is lower on intensively managed pastures compared to extensively managed pastures (Newbold et al., 2015). It was further estimated that land use and related pressures had already reduced local richness by an average of 13.6% and total abundance by 10.7% between 1500 and 2005 (Newbold et al., 2015). Similar numbers were found by Murphy and Romanuk (2014) in their global meta-analysis, who used species richness as a measure for the magnitude of biodiversity change. They identified land use change as having the largest impact on biodiversity decline, leading to a decrease of 24.8% on average. As the transformation of the attributes of the Earth's surface is reaching a scale and rate that is unprecedented, it is crucial to get an understanding of the feedback loops between land use intensification, change in biodiversity, and functional consequences to be able to evaluate the tolerance of ecosystems to human activities (M. Fischer et al., 2010; Weisser et al., 2017). In this context, characterizing the soil biodiversity with respect to ecosystem functioning and functional traits, and how they are responding to expeditious environmental and anthropogenic change, has become a major focus of ecological research (Hooper et al., 2005; FAO, 2020; Van Meerbeek et al., 2021).

1.1.7 The soil microbiome

The belowground biodiversity reservoir is largely defined by the soil microbiome, which is a standardized term describing microorganisms that are found in soil, including archaea, bacteria, viruses, fungi, protists, and invertebrates (Delgado-Baquerizo et al., 2019; Fierer, 2017). Soil microorganisms are critical to global nutrient cycling and contribute to greenhouse gas emissions, mineralization processes, organic matter decomposition, and can also promote plant health (Dominati et al., 2010; Fierer, 2017; Fierer & Jackson, 2006). The community composition in soil has been shown to directly control the rate of metabolic processes in soils (Bodelier et al., 2013; Strickland et al., 2009), including nitrogen fixation, methanogenesis, nitrification, or denitrification (Paul, 2006), which makes the soil microbiome of great ecological and economic importance. Soil conditions, however, can affect soil chemical properties and redox potential, which in turn influences the soil microbiomes that rely on these pathways for growth (Paul, 2006). For example, carbon and nitrogen concentration (de Vries et al., 2012), moisture (Brockett et al., 2012; Cruz-Martínez et al., 2009), the soil matrix and its formation of aggregates with clay, silt, and sand particles (Daniel, 2005) have been shown to lead to changes in microbiome composition and diversity. This in turn may cause a feedback loop on the aboveground biota. In fact, several studies have found that increased belowground species richness and phylogenetic diversity lead to increased biomass production in experimental grasslands (e.g. Bengtsson et al., 2019; Bullock et al., 2007; Tilman et al., 2001). Moreover,

there is growing evidence that the immense diversity of the soil microbiome contributes significantly to shaping aboveground biodiversity and grassland ecosystem functioning (Bardgett & van der Putten, 2014). Furthermore, ecosystem functioning has been shown to depend on trophic interactions between above- and belowground biota, biogeochemical cycling, and plant-soil feedbacks (Bakker et al., 2019). In this context, numerous studies have shown that plants are closely associated with microorganisms that are recruited from the surrounding soil (Kowalchuk et al., 2002). The majority of microbes are critical for ecosystem functioning and are known for their positive interactions with plants (Berg et al., 2020). Recent studies even suggest that plant roots can be considered soil organisms due to their close symbiotic relationships with soil components and microorganisms (Bardgett et al., 2014; Caruso et al., 2019; Coleman et al., 2017; FAO, 2020). However, despite the underlying significance, the complex relationships between the different biodiversity levels of above- and belowground communities that contribute to the concept of ecosystem multifunctionality and stability, remain elusive. Therefore, it is of great importance to gain insight into the close interactions between plants and their associated microbes and to determine their feedbacks to anthropogenic factors.

1.2 Plants and their microbiome

1.2.1 Plants as holobiont

Plants are inhabited by various microorganisms, including bacteria, fungi, archaea, protozoa, and viruses, which are commonly described as the “plant microbiota” (Trivedi et al., 2020). Many years of research have demonstrated that these complex co-associations with plants are key drivers for the plant’s health, productivity, community composition, and thus ecosystem functioning (Bever et al., 2010). The added ecological functions provided by the associated microbiota are essential for the host’s ability to adapt to various environmental changes and conditions (Berg et al., 2014). The nature of these interactions has given rise to the concept of so-called meta-organisms or holobionts (Simon et al., 2019; Zilber-Rosenberg & Rosenberg, 2008). All multicellular organisms exist as holobionts or meta-organisms, which are comprised of the host and its synergistic and antagonistic interdependence with various microbial species. Consequently, the plant microbiota that colonizes different host compartments is viewed as an additional reservoir of genes and functions for their hosts (Berg et al., 2020; Zilber-Rosenberg & Rosenberg, 2008). Moreover, the holobiont is considered as an inseparable functional unit that underlies the principles of co-evolution and co-selection (Figure 1; Jones, 2013; Theis et al., 2016). The analysis of plant microbiomes therefore involves linking the community of associated microbiota with their hosts’ physiology and functioning as the interplay of both maintains the functioning and fitness of the holobiont (Berg et al., 2016). Within this holistic concept, diseases are correlated with dysbiosis, a result of diversity loss that consequently alters the

natural composition of microbes. This leads to a microbial imbalance and in turn provides an advantage to the emergence and spread of pathogens through cascading effects on the hosts' immune system (Berg et al., 2017). Therefore, microbial diversity and their natural balance is a key factor in preventing diseases in plants (Berg et al., 2020). The co-evolution of the synergistic host-microbe interaction has led to a specific establishment of associated microbes and the extent of this specificity is influenced by many factors and varies across plant individuals (Berg et al., 2020; Theis et al., 2016).

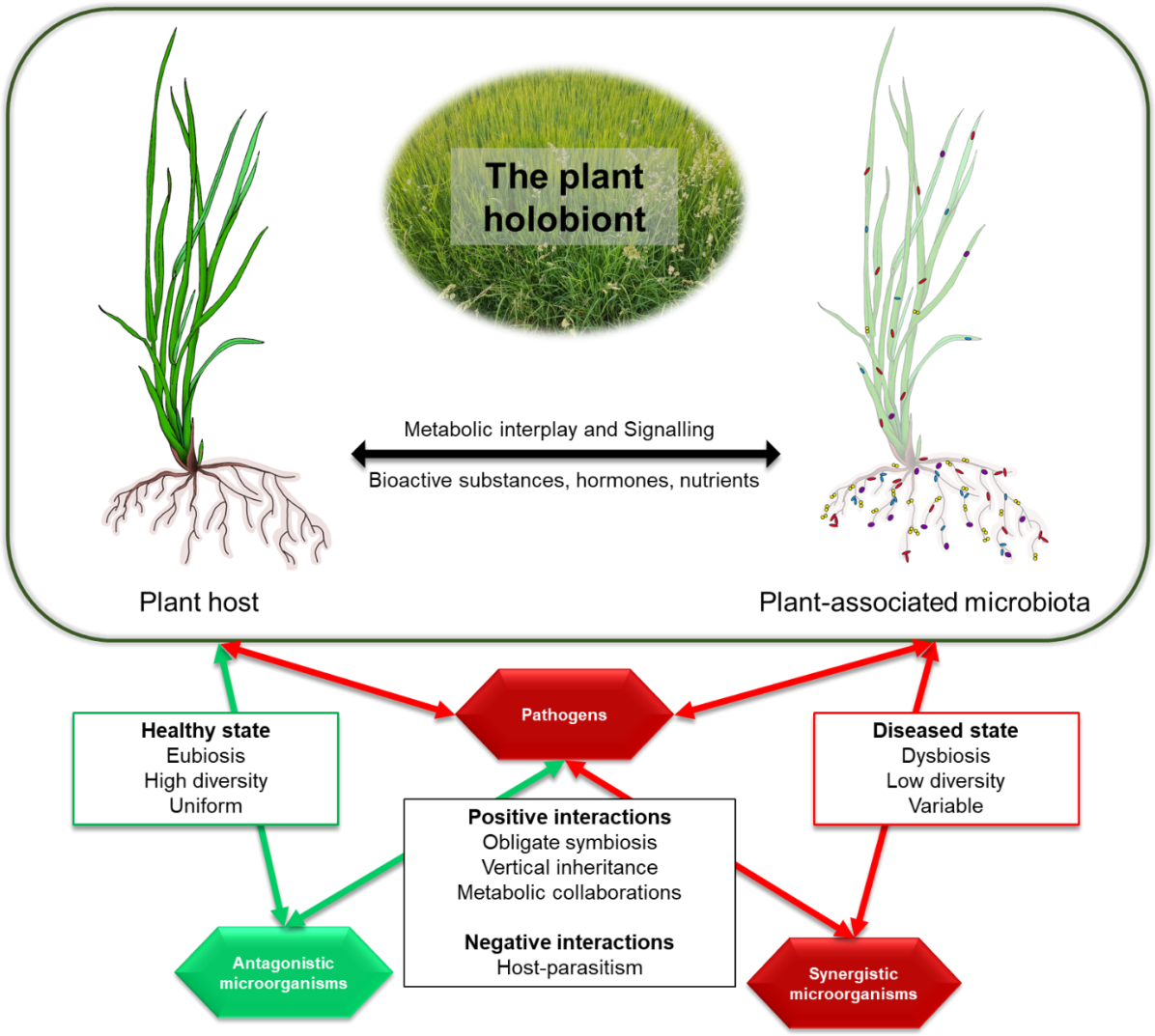

Figure 1: The plant holobiont

Illustration of the plant holobiont concept, the interplay between the plant, its associated microbiota and key interactions amongst each other. Within this holistic approach, the plant host and its associated microbiota are assumed to have co-evolved as a functional entity. While the diseased state is characterized by dysbiosis, low microbial diversity, and the variability of the respective microbiota (the "pathobiome" state), the healthy state is accompanied with eubiosis, high microbial diversity, and the uniformity of associated microorganisms. Microbes can act as antagonists or synergists towards pathogens and

depending on their phenotype may be beneficial or pathogenic (adapted from Berg et al., 2017 and 2020).

1.2.2 The rhizosphere

The rhizosphere represents the active soil layer surrounding the roots, which is directly influenced by root secretions and soil microorganisms (Berendsen et al., 2012). It is characterized by high bacterial abundances and activities, and is considered the most complex ecosystem on earth (Berg et al., 2014; Mendes et al., 2013). Microorganism-driven carbon sequestration in the rhizosphere allows organic material and compounds to migrate into subsoil, which is important for ecosystem functioning, nutrient cycling, and productivity in terrestrial environments (Berg et al., 2017). As bacteria are the predominant and most diverse form of life in soil that are essential for soil ecology (Sikorski, 2015), they were selected as the focus of the current study. Bacteria residing in the rhizosphere secrete a variety of plant beneficial compounds, such as phytohormones (e.g. auxins, gibberellins, ethylene, indole-3-acetic acid), cellulase, pectinase, superoxide dismutase etc., that are essential for plant growth, as well as the protection against environmental stressors and plant pathogens (Sahoo et al., 2018). The degradation of cellulose in high-organic-matter soils and the decomposition of pectin is widespread among bacterial communities residing in the rhizosphere (Turner et al., 2013). Therefore, the roots of terrestrial plants are the primary site for plant-bacterial interactions and were suggested as a protective shield against soil-borne pathogens (Berg et al., 2014; Sánchez-Cañizares et al., 2017). In addition to this, many bacteria can solubilize phosphorus-containing minerals, which leads to an increase of its bioavailability. Nitrogen-fixing bacteria provide a source of nitrogen for the host (Turner et al., 2013).

Microbial community composition in the rhizosphere is mainly shaped through “rhizodeposits”, a variety of carbon- and nitrogen-rich compounds that are released by the roots into their surrounding environment. These include root cells, plant cell wall polymers, such as cellulose and pectin, mucilage, soluble lysates, volatile compounds, and root exudates (Middleton et al., 2021; Turner et al., 2013). Root exudates are rich in chemical metabolites like flavonoids, amino acids, carbohydrates, phenolic compounds, and organic acids, which have been reported to be a major driving force in shaping microbial diversity and activity on plant roots (Chagas et al., 2017; Mendes et al., 2013). Differences in plant root exudates play a crucial role as they act as both chemo-attractants and repellents to which bacteria are particularly sensitive, which in turn causes a host species-specific bacterial colonization (Berg et al., 2017). Lundberg et al. (2012) revealed that bacterial composition in the rhizosphere is comprised of a subset of bacterial communities in the surrounding bulk soil. Biochemicals released by the roots are sensed through chemotaxis to initiate colonization via the use of flagella to move

towards the plant. As a result, some microorganisms may attach to the root surface and form a biofilm (Trivedi et al., 2020). In turn, some bacterial species may penetrate the roots of plants they are adhered to at root cracks, elongation zones, root tips, and below the root hair zone to become an endophyte (Mercado-Blanco & Prieto, 2012). Cracks or wounds may occur at the emergence points of lateral roots, or they may result from microbial or nematode activities (Reinhold-Hurek & Hurek, 2011).

1.2.3 The endosphere

“Endophytes” are microorganisms that colonize the plant inter- and/or intracellular tissue (endosphere) for at least part of their life cycle without causing apparent disease (Schlaeppi & Bulgarelli, 2015). However, despite their broad presence in many ecosystems, their mode of action, ecology, and evolution are poorly understood. The lack of a functional definition for endophytes implies that in addition to commensal symbionts, endophytes may also include latent pathogens, latent saprotrophs, or mutualistic associations (Fesel & Zuccaro, 2016; Hardoim et al., 2015). Hence, Hardoim et al. (2015) suggested that endophytes are defined by the habitat they colonize and not by their function, which means members of the endosphere comprise beneficial, neutral, and pathogenic bacteria. However, the nature of plant-bacterial interaction depends on numerous environmental parameters and biotic interactions (Brader et al., 2017). Several species-specific plant endophytes do not show any harmful effects, however, when tested on other plants, they are considered pathogenic. For example, *Pseudomonas* spp., frequently reported to have plant-beneficial effects, has been shown to be detrimental to leatherleaf ferns under certain conditions (Kloepper et al., 2013). Nonetheless, the capability of endophytes to successfully enter plant inner tissues have exposed their importance in agricultural practice for biocontrol of phytopathogens, plant growth promotion, and the production of biotechnological or pharmacological compounds (Sharma et al., 2017). In general, endophytic bacteria can have different life strategies in accordance with their intimacy degree with associated plant host. Whereas endophytes that require plant tissues to complete their life cycle are referred to as “obligate”, associated bacteria that mainly thrive outside plant tissues and only sporadically enter the plant endosphere are termed “opportunistic”. The intermediate group, the so-called “facultative” endophytes, represents the majority of endophytic microorganisms (Hardoim et al., 2015). Facultative endophytes live in soil, consume nutrients provided by the plant, and enter plants through systemic colonization when the opportunity arises (Hardoim et al., 2008). Bacteria residing within the plant endosphere were found in almost all plants studied to date and all plant compartments including roots, stems, leaves, seeds, fruits, and tubers (Rosenblueth & Martínez-Romero, 2006). With the emergence of high-throughput

technologies, such as next-generation sequencing, cultivation-independent analyses permitted the facilitation of large sample sizes and provided deep insights into microbial community compositions in numerous host plants. Among those were the model plant *Arabidopsis thaliana* (Bulgarelli et al., 2012; Schlaeppi et al., 2014), grapevine (Bruisson et al., 2019), barley (Bulgarelli et al., 2015), potato (Buchholz et al., 2019), rice (Ikeda et al., 2010), and wheat (Robinson et al., 2016). Even though a remarkable diversity was found within those studies, the plant endosphere is dominated by only a few bacterial phyla (mainly Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes), which account for 96% of the total number of endophytic procaryotic sequences suggesting a very selective niche (Hardoim et al., 2015). Despite this taxonomic overlap, distinct bacterial compositions are found among plant species (Berg & Smalla, 2009).

Though endophytes have been shown to be vertically transmitted via seeds (Barret et al., 2015), the main source for a species-specific attraction of bacteria is the rhizosphere, as it represents the largest reservoir of microbial diversity. Successful endophyte colonization requires compatible interactions between plant and microbe. Plant roots provide an attractive, nutrient-rich niche by providing easily degradable carbon, a protected environment, and physical structures for colonization (Berg et al., 2016; Kowalchuk et al., 2002). In return, root-associated bacteria positively influence plant growth by supplying nutrients like nitrogen or phosphorus and protection against phytopathogens (Berg et al., 2016; Reinhold-Hurek et al., 2015). However, the mechanism of environmental acquisition and composition dynamics of associated bacteria is poorly understood (Middleton et al., 2021). Plant-microbe interactions, bacterial abundance, and community composition are strongly susceptible to environmental conditions. Therefore, understanding how they are influenced by environmental changes is crucial for predicting disease outbreaks, arranging effective symbiotic and biocontrol agents, and designing resistant crop plants with increased resilience to current and future climate change (Cheng et al., 2019).

1.2.4 Heterogeneity of microbial communities across plant individuals

Numerous studies have been published over the last decade that describe the diversity of bacteria associated with different host plants, linking a number of important plant phenotypes to microbial variation (e.g. Bulgarelli et al., 2012; Fitzpatrick et al., 2018; Yang et al., 2017). For instance, the host identity plays a crucial role in assemblage patterns of associated microbiota, which can even lead to differences between plants that grow in close proximity to each other (Samad et al., 2017). Phylogenetically distant plants are likely to have a greater variation in associated microbiome compositions, suggesting that plant phylogeny plays a role in defining the plant-associated bacterial patterns (Bouffaud et al., 2014). Plant species are reported

to shape both the identity as well as diversity of bacterial communities (Dastogeer et al., 2018; Manter et al., 2010), indicating that host plant species evolved traits that determine the composition of its associated microbiota (Fitzpatrick et al., 2018). Another aspect influencing the plant-associated community composition is the genetics of the host plant. Several studies demonstrated that associated bacterial patterns differ between genotypes of a particular plant. Cultivar-specific effects on plant-associated communities have been reported for barley, sweet potato, wheat, pea, and oat (Bulgarelli et al., 2012; Marques et al., 2014; Turner et al., 2013; L. Yang et al., 2017). Bulgarelli et al. (2015) even suggested that genotype accounts for about 6% of the variation of associated microbes in the plant-root surrounding soil, the rhizosphere. However, studies on genotype-dependency were mainly conducted under greenhouse conditions, where key factors contributing to plant performance were kept optimal and only one factor was changed. This is supported by the results of Wagner et al. (2016), who found that under natural conditions plant genotype is only marginally relevant to the establishment of root-associated bacteria. Furthermore, the different plant compartments contribute to the formation of a distinct microbiome. The root-adhering soil and the different plant tissues of a plant harbor different community compositions, due to different adaptation strategies, and conditions they are exposed to (Dastogeer et al., 2020; Edwards et al., 2015). Microbes colonizing the phyllosphere (above-ground surface of the plant), for example, need to adapt to different conditions compared to microbes colonizing root-adhering or internal compartments, as they are exposed to seasonal weather fluctuations and surface tissues usually have a lower nutritional value (Lindow & Brandl, 2003). In contrast, root-associated bacterial assemblies are largely recruited from the surrounding soil. Through secretion of carbon compounds and phytochemicals, plant-influence patterns are distinct at different plant developmental stages, with low root exudation at the beginning of the vegetation period, an increase during growth peaks, and a high input of plant litter biomass during senescence (Chaparro et al., 2013). This, in turn, imposes selective pressure on bacterial community assemblages and creates a specialized microenvironment for specific bacterial groups (Chaparro et al., 2014). Investigation on the model plant *Arabidopsis thaliana* L. showed that levels of cumulative secretion of sugars and sugar alcohols were increased in early developmental stages and decreased throughout plant growth. In contrast, levels of cumulative secretion of amino acids and phenolics were reported to increase over time (Chaparro et al., 2014). Furthermore, root system architecture is genetically programmed throughout developmental stages leading to differences in the acquisition of soil resources (Canarini et al., 2019). Other plant-age related changes of associated bacteria may be root growth, physiology, and morphology, as these parameters have been shown to affect root exudation patterns, which in turn exerts selective pressure on soil-derived bacteria (Dastogeer et al., 2020).

The interdependence between the plant and its associated microbiota has led to the assumption that selective forces of evolution do not only impact the plant genome itself, but the entire plant holobiont and acquired heritable traits are made available through vertical transmission of valuable functions provided by associated microbes to their hosts (Hardoim et al., 2015). In this regard, factors contributing to selective enrichment of microorganisms were addressed by several studies. As plants heavily depend on their surrounding soil habitat, the majority of research on plant health benefits focuses on the rhizosphere (Berg et al., 2014).

1.3 Drivers of changes of microbial diversity at the plant-soil interface in grassland ecosystems

Beyond the plant host, the associated bacteria are interconnected with their surrounding ecosystem (Berg et al., 2014). Hence, to get a better understanding of how associated bacterial abundances and community composition are responding to environmental changes, it is important to take a deeper look at the conditions they are exposed to. Soil environments are difficult to characterize microbiologically due to their complexity, as bacteria are influenced by a multitude of factors (Bottos et al., 2020; Fierer, 2017). Thus, identifying drivers and relationships of the underlying interactions between soil and plant-associated bacteria is even more difficult due to their complex interplay (Cheng et al., 2019; Fierer, 2017). With regards to different land management regimes on grasslands, factors are generally classified as (a) abiotic, like climate conditions and chemical and/or soil type (non-living part), (b) biotic, which include above- and belowground biota (living part), and (c) anthropogenic factors (human introduced), such as fertilization, grazing, or mowing. In a given plant-bacteria interaction especially, it is expected that an environmental condition would be forced on both the plant and the associated bacteria (Cheng et al., 2019). Host-associated bacteria have already been shown to be able to mitigate biotic and abiotic stressors through their ability to adjust to changes rapidly (Berg et al., 2017). However, while pathogen defence mechanisms are well understood (Berg et al., 2014), abiotic stress protection mechanisms, such as alleviating the impact of anthropogenic disturbances, is just at its infancy. Although poorly understood, environmental factors that influence host-microbiome interactions are vital to understanding the evolution and ecology of these symbiotic relationships (Fitzpatrick et al., 2018). Several individual variables are introduced in the following section, although complex interactions could exist that includes modulation of these variables by higher-level factors. These in turn may directly or indirectly interfere with above- and belowground biota and thus have an influence on plant-associated bacteria (Hinsinger et al., 2009). A selection of drivers that might be of relevance for the soil and plant-associated bacterial communities in grasslands is described below and schematically depicted in Figure 2.

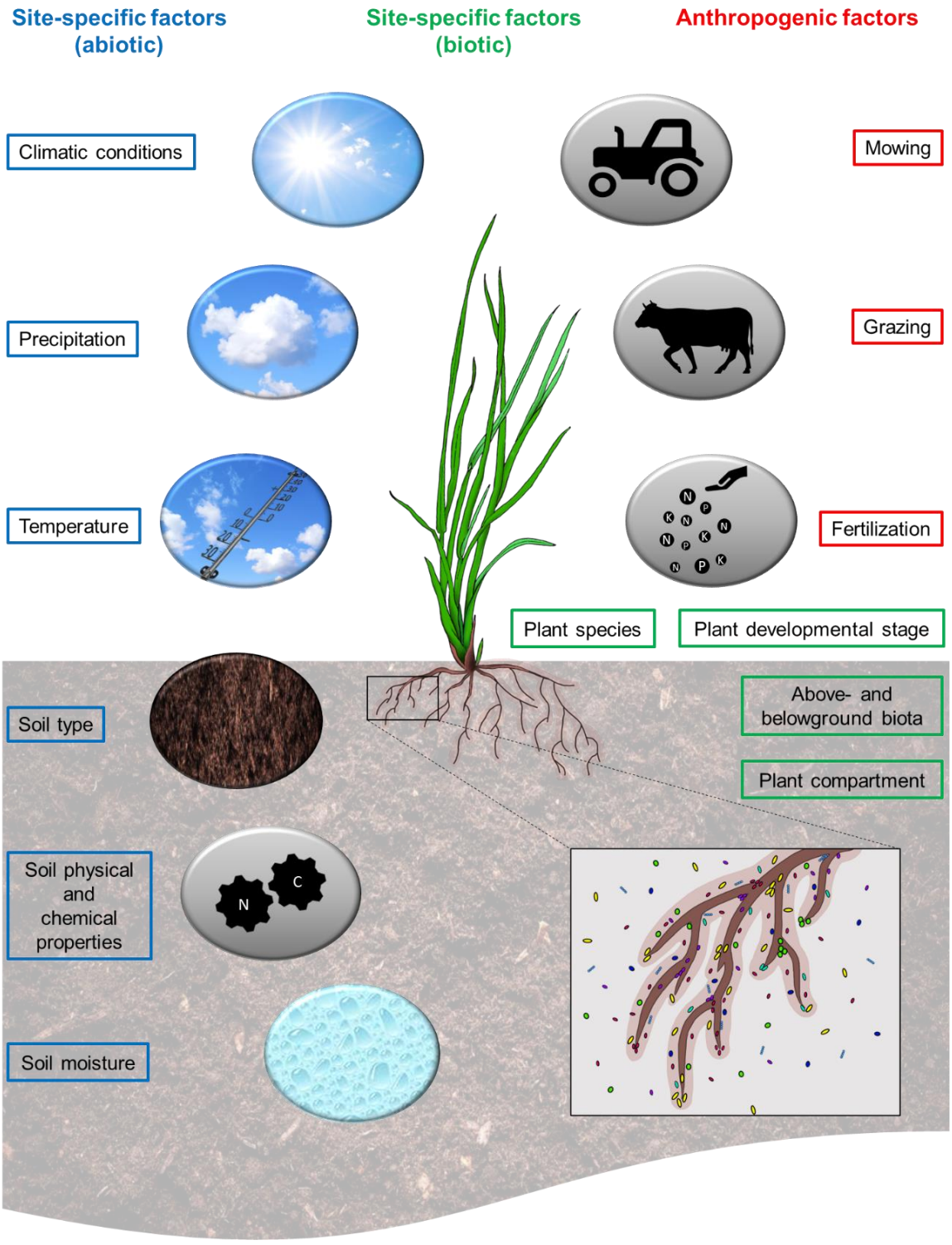

Figure 2: Drivers of the composition of plant-associated bacterial communities in grassland ecosystems

Summary of a selection of environmental variables that influence the composition of plant-associated bacteria, divided into site-specific factors (biotic and abiotic) and anthropogenic factors. The selection of factors belonging to these environmental variables is exemplary. The classification is indicated by a color code: site-specific abiotic (blue), site-specific biotic (green), anthropogenic (red).

1.3.1 Site-specific factors

Plant-associated microbes are mainly recruited from its surrounding soil (Berg et al., 2014). Hence, one of the most important factors for bacterial community structure is the soil they grow in. Various soil parameters have been reported to influence the associated bacterial communities of plants. Among those are soil type, soil pH, C:N ratio, as well as available phosphorus (Dastogeer et al., 2020). Several studies provided insight into effects of soil type on the bacterial community composition in the rhizosphere (Bulgarelli et al., 2012; Lundberg et al., 2012; Schreiter et al., 2014). Dombrowski et al. (2017) reported that soil type is causing variation in root-associated bacteria of up to 15% and influences the composition to a higher extent than the host plant. Similarly, soil chemical properties influence nutrient availability and associated processes in soil, plant productivity, and organic matter decomposition (Ranjard et al., 2013). Studies at local to continental scales have found that soil properties significantly influence soil bacterial community structure and diversity, while plant communities and land use act as secondary correlates (Plassart et al., 2019). As there is no physical separation between bulk soil and the rhizosphere, these factors are of particular importance to the plant-bacterial interactions in the rhizosphere compartment.

The effects of environmental change are complex and cannot be reduced to a single factor such as CO_2, temperature, or precipitation (Bottos et al., 2020; Fierer, 2017; Gschwendtner et al., 2015). Elevated temperature, for example, has been shown to influence the kinetics of biochemical processes in the soil (Avrahami et al., 2002), but it can also mediate changes of plant-immune responses, such as the suppression of effector-triggered immunity, which inversely has an impact on the plant-associated communities (Cheng et al., 2019). In addition to this, climatic conditions can contribute to alterations in soil moisture, which affects redox potential of the soil. Climatic conditions fluctuate continuously and may influence the plant and soil processes throughout the different seasons. Balanced soil moisture is essential to numerous processes. It affects nutrient availability by facilitating nutrient solubility and transport, as well as mineralization rate, which in turn affects enzymatic activity in the soil (Stempfhuber et al., 2016). As nutrient acquisition is one of the most important drivers for plant-bacteria interaction, nutritional state of the plant and the availability of nutrients are expected to have significant effects on the plant-bacteria interactions (Cheng et al., 2019). Additionally, soil water content can greatly impact numerous aspects of the plant-bacteria interactions through a direct effect on the plant such as the regulation of the phytohormone abscisic acid (ABA). This in turn has an influence on the closure of plant stomata and consequently reduces bacterial entry through these (Melotto et al., 2006; J. K. Zhu, 2016) or causes a shift in plant metabolites (J. K. Zhu, 2016), which could have an adverse effect on the associated bacteria. In fact, soil moisture was shown to change the bacterial diversity within the rhizosphere and root endosphere (Xu et al., 2018; L. Yang et al., 2020).

As the diversity of organisms is tightly linked at different hierarchical levels, there are a multitude of direct or indirect interactions between plant-associated bacteria and other organisms. On the one hand, these might be of cooperative nature, which are either mutualistic (both species benefit from the interaction) or commensalistic (one partner benefits from the interaction whereas the other is not negatively impacted; Schlechter et al., 2019). On the other hand, plant-associated bacteria may also compete directly or indirectly with other microbes either through various competition mechanisms or predation and parasitism. Competitive relationships are associated with adverse effects for at least one species, which may be due to exploitative or interference competition. Exploitation or passive competition is the competition for certain resources through enhancing utilization of limiting nutrient resources by altering their metabolic regulation or restricting access to nutrients (Schlechter et al., 2019). These direct or indirect interactions may in turn have an effect on the composition of the plant-associated bacteria.

1.3.2 Anthropogenic land management factors

Land use intensification is considered one of the most important anthropogenic causes contributing to changes in biodiversity and ecosystems (Allan et al., 2015). In European grasslands, intensification involves higher fertilization rates, increased livestock densities, and mowing frequency (Manning et al., 2015). Land use management activities have been shown to affect the physical, chemical, and organic soil properties, which do not only alter the soil structure, amount, and quality of nutrient availability, but also affect nutrient cycling in soil causing a feedback loop on above- and belowground biota (Felipe-Lucia et al., 2020; Herold et al., 2014; Sorkau et al., 2018). On intensively managed grasslands, forage quality and biomass are generally preferred at the expense of multitrophic diversity and other ecosystem functions, as it is less dependent on soil functions, but can be determined by the amount of fertilizer application (Felipe-Lucia et al., 2020). Extensive land use in this matter refers to unfertilized or unmanaged soil. Fertilization, grazing, and mowing may play an important role for the establishment of plant-associated bacteria within grassland ecosystems and were chosen as the major components to determine land use intensity on the sampling sites for this study.

Removing the aboveground biomass by mowing is a globally common practice in grasslands. As mowing can impair the availability of light for small, subdominant species, it can disturb the plants' competition for nutrient resources and change the quality and quantity of root exudates (Kuzyakov & Xu, 2013; Patra et al., 2006; Wolters et al., 2000; Y. Zhang et al., 2017). Following mowing, root exudation and ammonia levels rise, leading to the enrichment of bacterial activity and biomass (Hamilton & Frank, 2001; Herold et al., 2014; Kuzyakov & Xu, 2013; Le Roux et al., 2008). Thus, mowing in general results in beneficial changes to belowground bacterial

communities. Furthermore, remaining plant litter input may positively impact decomposition processes, as changes in plant species composition and exudation can be associated with mowing, which in turn may impact the root-associated bacterial communities (Patra et al., 2006; Zhang et al., 2017).

Numerous ecological effects on above- and belowground biota can derive from grazing through herbivory, physical impact, and deposition. Grazing affects aboveground plant community composition through selective herbivory or through differential tolerance of plants to grazing (Furbish & Albano, 1994). Continued selective grazing can reduce the competitive ability of grazed plants. Trampling can further injure plants indiscriminately and reduce their ability to compete and reproduce within the plant community (Fleischner, 1994; Huntly, 1991), thus affecting plant metabolism and root exudation patterns (Herz et al., 2018). Moreover, trampling can disturb soil bacterial communities by influencing soil compaction and destroying soil crusts which in turn changes soil structure, nutrient cycling, biomass production, and water infiltration (Bardgett & Wardle, 2003; Patra et al., 2006). In addition to this, organic components of faeces and urine from grazing animals can build soil organic matter reserves and increase phosphorus availability in soil, which enhances cycling of nitrogen and carbon (Patra et al., 2006; Ritz et al., 2004). All these changes of chemical and physical properties of the soil and to plant community composition, plant metabolism, and root exudation patterns may have a direct influence on belowground communities and thus exerts selective pressure on plant-associated bacteria in the rhizosphere and endosphere (Steffens et al., 2009; Yang et al., 2021). Several studies have identified grazing-induced changes of bacterial community composition and diversity in soil and rhizosphere (Kohler et al., 2005; Patra et al., 2005; Xun et al., 2018), however, studies on changes of bacterial endophytes and their response to grazing have been rare (Yang et al., 2021).

The application of mineral and organic fertilizers has become an essential component of modern agriculture as it can increase crop yield through providing nutrients such as nitrogen, phosphorus, and potassium (de Souza et al., 2015). Especially in unfertilized soils, plants may rely on nitrogen derived from bacterial transformation processes such as mineralization and nitrogen fixation. Thus, they play a significant role in alterations of soil chemical properties and affect soil functioning, quality, and improve nutrient availability for plants (Francioli et al., 2016). Despite the positive effects on crop productivity, contrasting effects of fertilizer application have been found with respect to soil microbial growth and activity. While most long-term field experiments found a positive correlation between mineral fertilization and soil bacterial biomass (Geisseler & Scow, 2014), other studies based on short-term experiments found opposite results (Lazcano et al., 2013; Roberts et al., 2011). Moreover, contrasting effects have been reported in response to mineral fertilization regarding soil bacterial activities in grassland soils (Nannipieri et al., 2012). Comparisons between the effects of mineral and organic fertilization

on soil communities have shown that organic manure fertilization results in higher soil bacterial biomass and maintains different community patterns (Esperschütz et al., 2007; Lazcano et al., 2013; Lentendu et al., 2014; Nõlvak et al., 2016). As soil bacterial community composition and enzymatic activity depend on fertilization application, it is evident that bacteria being in the plant-soil interface may respond differently as well (Deng et al., 2021; Li et al., 2020; Zhu et al., 2016). In contrast, the influence on endophytic communities remains largely unknown, although, as plant surrounding soil has been shown to be the main source for their recruitment, the impact of fertilization on bacterial endophytes seems imperative (Amadou et al., 2020).

1.3.3 Land use intensity

Investigation of the relationship of land use management and diversity of plant-associated bacteria may deliver contradictory results depending on the different site factors and anthropogenic land management regimes (Deng et al., 2021; Li et al., 2020). Furthermore, a multitude of regimes may be used to manage land, all of which may affect community composition and thus may impede comparability of the effects of land use. Yet, most studies use measures of land use that underlie categorical classifications, such as pastures versus meadows or fertilized versus unfertilized, which might be useful to certain comparisons or specific experiments. However, they do not account for a quantitative variation in land use intensities (Blüthgen et al., 2012). Furthermore, the intensity of mowing, grazing, and fertilization may vary in space and time, within and between years on particular grasslands, causing spatial and seasonal differences and thus may contribute to contradicting results (Blüthgen et al., 2012; Vogt et al., 2019), which severely limits studies that aim to quantify land use effects across existing landscapes (Blüthgen et al., 2012). These factors, however, do not exclusively influence the composition of plant-associated bacterial community patterns, but should be considered as a combination of several components of land use, as they often interact with each other, mostly nonlinearly and across existing heterogenous landscapes and determine the environmental conditions of a certain habitat (Blüthgen et al., 2012). Furthermore, biodiversity may be affected directly or indirectly by intensification. The mechanical force of mowing, for example, can directly impact the mortality of insects, whereas increased fertilization may have an impact on the plant community composition and in turn affect insect diversity (Vogt et al., 2019). Hence, land use can be considered a multi-layered and multi-dimensional interplay of anthropogenic activities and ecological processes with several influence measures, potentially interacting as direct and indirect impacts on biodiversity (Dullinger et al., 2021; Erb et al., 2013). In addition to this, little attention has been paid to long-term and in-depth assessments of management regimes, which altogether significantly restricts studies that aim to quantify land use effects

across existing landscapes (Vogt et al., 2019). The multi-dimensional nature of land use intensity in grasslands is rarely captured in categorical classifications, and thus is inadequately reflected in the majority of biodiversity research (Blüthgen et al., 2012; Dullinger et al., 2021). To get a more systematic and mechanistic understanding of the relationship between land use intensification and biodiversity in grasslands, detailed information on management regime is needed, which ideally is collected over several years (Vogt et al., 2019). Hence, a continuous measure for land use intensity was developed, the land use intensity index (LUI index), which includes mowing, grazing, and fertilization as the major components of land use (Blüthgen et al., 2012).

1.4 The Biodiversity Exploratories

In order to advance the knowledge on a large scale, a research platform for functional biodiversity research was established in 2006, the Biodiversity Exploratories (www.biodiversity-exploratories.de; Fischer et al., 2010). The Biodiversity Exploratories (further referred to as BEO), funded by the German research foundation (Deutsche Forschungsgemeinschaft, SPP 1374 Priority Programme), is a large-scale and long-term project to comprehensively examine the relationship between land use, biodiversity, and ecosystem functioning in real-world ecosystems. It serves as study regions for scientific working groups covering numerous research fields such as microbiology, zoology, and botany, and thus allows comprehensive interdisciplinary research (Fischer et al., 2010). A major aspect of this project is the thorough monitoring of land use on the study plots over several years to allow statistical comparisons across taxa, different land use types, or geographical regions across temporal variation in land management (Vogt et al., 2019). Systemically surveyed management types and frequencies are gathered as part of the design, including mowing, grazing, and fertilization on grassland sites, which is used as the basis of the integrated land use intensity index (LUI index; Blüthgen et al., 2012). As land use change is the major driver of changes of biodiversity and biological ecosystem processes and services, experimental plots comprising different levels of land use intensity have been included (Fischer et al., 2010; Sala et al., 2000). All experimental plots are within the three large-scale study regions: UNESCO Biosphere Reserve “Schorfheide-Chorin” (Brandenburg), the National Park Hainich and surrounding areas, i.e. “Hainich-Dün” (Thüringen) and the UNESCO Biosphere Reserve “Schwäbische Alb” (Baden-Württemberg) and distributed on a north-east to south-west gradient (Fischer et al., 2010). Each exploratory is subdivided into 50 grassland experimental plots of 50 x 50 m (EPs). A subset of 9 plots is used as very intensive plots (VIPs), plots where biodiversity and ecological processes are studied in detail or for studies that use too labor-intensive methods to be conducted on all EPs. Characteristics like the presence and year of the start of drainage and whether arable farming was practiced during

the 25 years before the project was started, i.e. between 1981 and 2006 (Vogt et al., 2019) are recorded. All evaluated information is made available to all participating researchers. Thus, the design of the BEO does not only provide a platform for the investigation of integral effects on biodiversity in grasslands, but it also enables a multidisciplinary data interpretation on a spatial scale and long-term nature. Hence, the research for this thesis was conducted as part of the framework of the BEO.

1.5 Open questions, research aims and hypotheses

Plant-associated bacteria have an undoubtedly significant impact on plant health, development, nutrition, and resistance against stress factors and therefore are crucial for ecosystem services and processes (Middleton et al., 2021). Due to its importance to the functioning of the plant holobiont, the host- and site-specific establishment of bacterial assemblages in endosphere and rhizosphere compartments have been vastly studied among different plant species. Despite the amount of data available, most studies on plant-associated interactions were performed under controlled, short-term and optimized conditions for host-plant growth, where only individual aspects were changed. Investigations are rarely conducted under variable and field-realistic conditions (Berg et al., 2017). Plants grown under natural or less favourable conditions might experience different effects attributed to their associated bacteria, as several environmental factors may interfere or vary in space and time, thus dynamically shaping the composition of plant-associated bacteria. Hence, there is a huge lack of knowledge regarding the driving forces on the establishment of bacterial assemblages within the rhizosphere and endosphere and the effects of a multitude of factors on their composition in natural environments.

Given that intensive land use management has led to an alarming decline in biodiversity over the last decades, understanding the processes and factors governing the dynamics of plant-associated bacterial assemblages in response to anthropogenic factors is crucial. The ability to reliably predict and control associated microbes in the environment would offer significant opportunities to help maintain the health and functioning of ecosystems and improve resilience to current and future climate change (Stegen et al., 2018). Though several components, which account for numerous stability mechanisms and contributions to buffering the effect of environmental fluctuations on ecosystem functioning have been identified (de Bello et al., 2021), the complexity of specific plant-microbe interactions represents an enormous challenge and needs more thorough investigations. Although different contributions and response patterns of plant-associated bacteria to fertilizers or specific land management regimes have been identified (Li et al., 2020; Zhu et al., 2016), real management situations are not reflected appropriately due to the overlapping effects and multifactorial nature of land use intensity. No study

has investigated the long-term impact of land use intensity attributed to a continuous measure including several components and the feedback loop on plant-associated bacteria to reflect real world scenarios. It remains to be clarified whether LUI can be considered a general driver of changes in bacterial composition dynamics through shaping substrate conditions and properties.

Numerous studies suggest that despite the assemblage dynamics of plant-associated bacterial communities, plants may harbor a core of bacterial taxa that are present regardless of disturbing parameters, environmental conditions, and plant developmental stage (Lundberg et al., 2012; Pfeiffer et al., 2017). Members of the core might be potentially crucial for the fitness of the plant holobiont by establishing a healthy community composition that supports nutrient uptake and mitigates stress responses (Berg et al., 2017). Loss of those keystone taxa may induce dysbiosis, therefore significantly reducing plant fitness and the displacement by other plants, which in turn may trigger shifts in biodiversity patterns. The consequences of LUI for the formation of a species-specific establishment of keystone taxa remains elusive.

Hence, the main focus of this study was to identify plant-associated bacterial compositions on high versus low land use intensity measures (LUI). In this respect, bacterial relative abundance was assessed, then used to identify diversity and community composition in the endosphere, rhizosphere, and bulk soil from samples collected within three different plant developmental stages, representing the vegetative, flowering, and senescence plant developmental stage. Orchard grass (*Dactylis glomerata* L.), a perennial forage grass, was selected as a model plant because it is considered to be one of the most important grasses for grazing and hay production (Costa et al., 2016) and exhibited the highest continuity at the investigated sites. The research was conducted within the framework of the BEO, which allowed the investigation on a field-realistic environment and the effects of long-term, quantitative, and continuous LUI measures on the composition dynamics of associated bacteria. Different plant developmental stages permitted the description of core taxa (species present irrespective of the plant stage) on high versus low LUI and in different compartments. Moreover, I assumed that the comparison of the core taxa of different LUIs would enable the identification of keystone taxa, which are bacteria that are found regardless of site-specific conditions, plant environmental stage, and anthropogenic factors. Thus, insights can be gained into how land use intensity affects the variability of bacterial community composition over time and identify important drivers for a species-specific bacterial community composition under real world conditions.

In this context, I hypothesized that:

(i) The effects of LUI on bacterial diversity and community composition are most pronounced within the bulk soil and the rhizosphere. In contrast, the influence on root endophytic communities is less pronounced and mainly mediated by the plant. It is expected that bacterial diversity will be higher on low compared to high LUI measures in all investigated compartments as multitrophic diversity is strongly favored on low LUI levels due to a higher dependence on soil functions.

(ii) Bacterial composition within the endosphere is distinctive due to specific attraction and only few bacterial taxa being able to penetrate the plant roots. The community composition in the rhizosphere and bulk soil however is more similar due to no spatial delineation.

(iii) The major driver of shifts in community composition is LUI by shaping soil properties and changing the nutrient availability, which causes a feedback loop on bacterial diversity in the root endosphere.

(iv) A set of shared core taxa will be present throughout all plant developmental stages but dependent on LUI measures. The structure of shared bacterial communities will be more complex and variable throughout the different plant developmental stages on low LUI levels. Conversely, a collection of bacterial keystone taxa will be present across all samples independent of LUI levels, plant developmental stage, or other disturbing parameters, which are considered as a host-specific set of microbes potentially crucial for the establishment of a healthy community composition and essential to the functioning of the plant holobiont.

2 Material and Methods

2.1 Study site description

The experiments for this study were performed on selected plots of the UNESCO Biosphere Reserve “Schwäbische Alb” within the frame of the German Biodiversity Exploratories. Schwäbische Alb is located in the submontane and montane plateaus in the southwest of Germany (Figure 3a) and is covering an area of about 422 km^2 and includes 50 grassland experimental plots (EPs) with a size of 50 x 50 m.

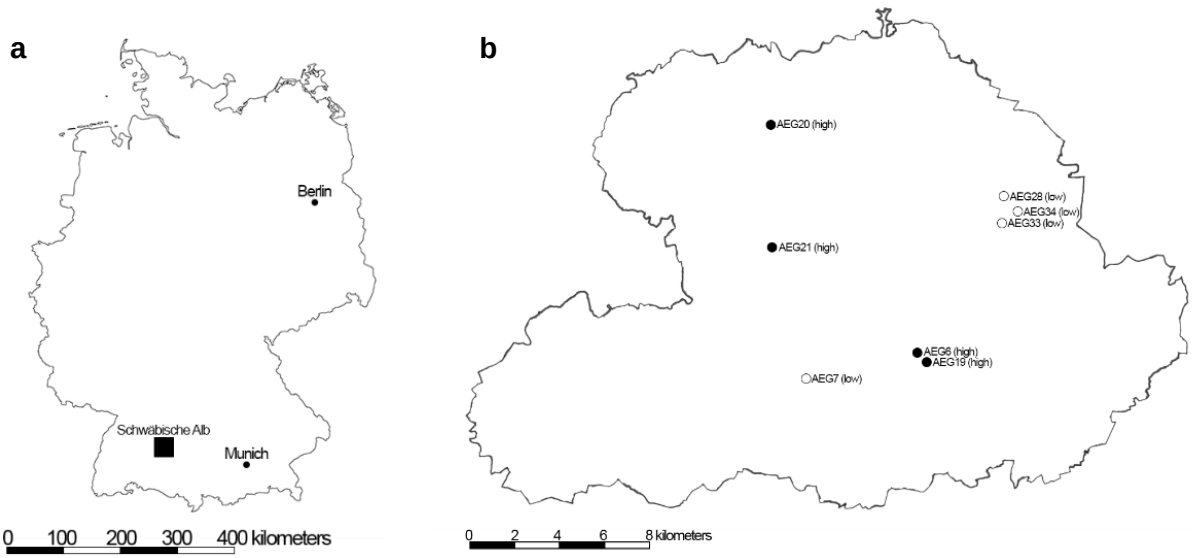

Figure 3: Overview of the study region “Schwäbische Alb”

(a) Schematic representation for the location of the exploratory “Schwäbische Alb” within Germany; **(b)** Location of the selected experimental plots within the “Schwäbische Alb” and the classification into high LUI (black dots) and low LUI (white dots).

Land use is represented by a variation of mowing regime, fertilization, and grazing management, ranging from hardly managed grasslands to highly fertilized and intensively managed meadows and pastures. EPs are classified into a land use intensity index (see next chapter). Monitoring units are installed on each plot measuring above ground temperature, humidity, soil surface temperature, soil moisture, and soil temperature. To prevent damage from livestock or other animals, these monitoring units are fenced. However, the remaining area is neither fenced nor separated from the surrounding area. Hence, the measurements do not interfere with the management that is applied on the surrounding grassland (Fischer et al., 2010). The mean annual temperature for the year 2015 has been determined as 9.9 °C and the mean

annual precipitation was 730 mm. Mean temperature and precipitation during sampling collection was 12.3 – 22.7 °C and 80.6 mm.

For this study, eight experimental sites were selected based on available data that was generated in the frame of the soil sampling campaign 2011 of the BEO using soil type, land use intensity index (2006 to 2010), plant available nitrogen, and phosphorus fractions in soil and were subsequently classified into high vs. low land use intensity (Table 1). The soil type of the selected experimental sites is described as Rendzic Leptosol (according to the FAO classification system). Soil texture of the plots was determined as follows: clay content was in the range of 423 – 637 g/kg soil, silt content in the range of 327 – 554 g/kg soil, and sand content in the range of 15 – 69 g/kg soil with exception of plot AEG7, showing differing values (clay: 385 g/kg soil, silt: 427 g/kg soil and sand: 188 g/kg soil; Table 1).

2.2 Land use intensity index

The land use intensity index (LUI index) is represented by three different management regimes that are weighted equally. Thus, it provides a unique design for studying bacterial communities and their responses to changes in management practices over time. The main components of land use intensity on the grassland plots of the BEO are mowing, grazing, and fertilization, ranging from unfertilized and extensively grazed sheep pastures over fertilized and mown cattle or horse pastures to intensively fertilized three-cut meadows or cattle pastures. Land use and management regime are evaluated yearly by interviewing farmers and landowners. Whereas mowing intensity is measured as the number of mowing events, fertilization intensity is calculated through the amount of nitrogen that is applied per hectare using organic (slurry or manure) or mineral fertilizers and grazing intensity is based on the number of grazing animals (standardized as livestock units) per hectare as well as the duration of grazing periods (Blüthgen et al., 2012). The LUI index is then calculated from the standardized mean of the corresponding model region and summed into an index of overall land use intensity using the values of mowing (number of cuts), fertilization (quantified in kg nitrogen ha^{-1}), and grazing (quantified in livestock units days of grazing ha^{-1} $year^{-1}$) per experimental plot (Blüthgen et al., 2012). The resulting values of LUI ranges used for the plot selection (based on the years from 2006 to 2010) are between 0.58 and 3.4 (Table 1; Plot ID's intensive LUI: AEG6, AEG19, AEG20, AEG21; Plot ID's extensive LUI: AEG7, AEG28, AEG33, AEG34; see Figure 3b and Table 1). The average LUI over several years (2006 to 2014) was used for the analysis of the data sampled in 2015.

Table 1: Experimental plot characteristics

Concentrations of $NaHCO_3$-Pi and Po (inorganic vs. organic sodium bicarbonate, labile/plant available phosphorus); NaOH-Pi and Po (inorganic vs. organic sodium hydroxide, moderately labile phosphorus); NH_4-N (ammonium-bound nitrogen) and NO_3-N (nitrate-bound nitrogen) given in mg^{-1} soil. Clay includes fractions <0.002 mm, Silt fractions between 0.002 – 0.063 mm and Sand fractions are between 0,063 – 2 mm in diameter.

PlotID	LUI index [2006 to 2010]	NO_3-N [mg^{-1}]	NH_4-N [mg^{-1}]	$NaHCO_3$-Pi [mg^{-1}]	$NaHCO_3$-Po [mg^{-1}]	NaOH-Pi [mg^{-1}]	NaOH-Po [mg^{-1}]	Clay [g/kg]	Silt [g/kg]	Sand [g/kg]	LUI
AEG6	2.47	5.41	3.37	25.02	21.31	240.62	653.01	588	380	32	high
AEG19	1.76	10.11	5.54	31.03	41.8	327.08	823.73	637	327	36	high
AEG20	1.94	3.96	0	39.53	23.77	246.07	773.21	597	334	69	high
AEG21	3.4	10.63	1.87	11.24	24.61	123.71	692.34	587	391	22	high
AEG7	0.58	0	3.81	1.6	1.45	18.04	166.56	385	427	188	low
AEG28	0.78	0.79	2.41	4.84	6.43	70.31	530.97	599	385	16	low
AEG33	1.12	0	2.44	4.52	10.18	47.7	426.83	597	388	15	low
AEG34	1.02	0.56	7.65	6.89	15.47	92.37	657.16	423	554	23	low

2.3 Sample collection

The sampling campaigns were carried out in May, June, and October 2015, which represented seasonal variations of the plant developmental stages. In May, *Dactylis glomerata* L. plants were in the vegetative state, in June, plants were in the reproductive state (production of seeds), and in October, plants were in the senescence state. In each experimental plot, samples were collected within a subplot of 1.5 x 1.5 m. Nine *Dactylis glomerata* L. plants without disease symptoms like leaf spots were excavated at a depth ranging from 10 to 15 cm with adherent rhizospheres and bulk soil. Soil that was shaken off the roots was defined as bulk soil. The remaining, closely adhering soil to the roots was defined as rhizosphere. Root samples with surrounding rhizosphere were collected separately from bulk soil, both in 5 ml tubes for DNA extraction. The samples were immediately frozen on dry ice and stored at -80 °C until further analysis. Above-ground biomass of the plant was harvested using sterilized scissors and immediately frozen at -20 °C. For soil carbon and nitrogen measurements, additional soil samples were sieved and stored at 4 °C.

2.4 Soil carbon and nitrogen content

For the determination of soil carbon and nitrogen content, an extraction protocol using 0.5 g of fresh soil and 0.01 M calcium chloride solution (1:2.5 soil to extractant ratio w/v) was performed. The extracts were shaken on a horizontal shaker for 45 minutes followed by centrifugation (2 minutes at 4500 x g) and filtered through a Millex HV Millipore filter, pore size, 0.45 µm (Merck, Darmstadt, Germany). Afterwards, water extractable organic carbon (WEOC) and nitrogen (WEON) were measured on a DIMA-TOC 100 (Dima Tec, Langenhagen, Germany). The same extracts were used to determine nitrate (NO_3-N) and ammonium (NH_4-N) through a continuous flow analysis with a photometric autoanalyzer (CFA-SAN Plus; Skalar Analytik, Germany).

2.5 Plant carbon and nitrogen content

To determine plant carbon and nitrogen content, above-ground plant material was dried at 65 °C for 2 days and then pulverized using a Tissue LyserII (Qiagen GmbH, Germany). Afterwards, 1.5 mg of the pulverized above-ground material was weighted into 3.5 × 5 mm tin capsules (HEKAtech GmbH, Wegberg, Germany) and subsequently measured for carbon and nitrogen contents with the Elemental-Analysator ‘Euro-EA’ (Eurovector, Milano, Italy).

2.6 Separation of rhizosphere compartment and surface sterilization of the roots

For the removal of the rhizosphere, *Dactylis glomerata* L. roots with adhering rhizosphere were transferred to a falcon tube containing 7.5 ml of 1x PBS improved with 0.02% Silwet (PBS-S; AppliChem, Darmstadt; Silwet L-77) and shaken at 180 rpm for 5 minutes. The procedure was repeated three times by transferring the roots to a new sterile 15 ml falcon tube containing 1x PBS-S. Following centrifugation, the PBS-S buffer was discarded and the remaining pellet was frozen in liquid nitrogen and stored at -80 °C.

After removing the rhizosphere, roots were submerged in sterile 1% Tween 20 for two minutes and then washed with pure autoclaved water. Subsequently, roots were incubated in 70% Ethanol for two minutes and then thoroughly rinsed in sterile distilled water three times. Afterwards, roots were surface sterilized in 5% sodium hypochlorite for ten minutes, washed eight times, frozen in liquid nitrogen and stored at -80 °C. Using the final wash water as a template, the efficacy of the disinfection process was confirmed by the absence of amplification of the 16S rRNA gene. In addition to this, 200 µl of the final wash water were plated onto NB-agar plates, incubated for 10 days at 28 °C and tested for growth of microbial colonies. This method was used, because microscopic investigation on ultrasonication and mechanical treatments revealed that they do not efficiently reduce rhizoplane community and may even damage plant tissue caused by sodium hypochlorite disinfection. Furthermore, surface sterilization adequately removes surface bacteria and therefore more accurately represents the community composition within the endosphere compartment (Reinhold-Hurek et al., 2015; Richter-Heitmann et al., 2016).

2.7 DNA extraction, library preparation and sequencing

2.7.1 Nucleic acid extraction

DNA was extracted using a phenol-chloroform-based method modified from Lueders et al. (2004). The extraction was carried out on surface sterilized roots, extracted rhizosphere, and collected bulk soil from all sampling sites and taken in May, June, and October. All samples were stored at -80 °C. To extract DNA within the root samples, they were frozen in liquid nitrogen and pre-homogenized in a TissueLyserII (Qiagen GmbH, Germany). 0.1 g of pulverized roots and 0.3 g of rhizosphere and bulk soil, respectively, were then homogenized in lysing matrix tubes E (MP Biomedicals, France) in a 120 mM sodium phosphate buffer (pH 8) and TNS solution [500 mM Tris-HCl pH 8.0, 100 mM NaCl, 10 % SDS (wt/vol)]. Afterwards, samples were centrifuged at maximum speed (16100 × g) for 10 min at 4 °C, the supernatant

discarded and successively mixed with an equal amount of Phenol/Chlorophorm/Isoamylalcohol (25:24:1 ratio, Sigma-Aldrich) and Chloroform/Isoamylalcohol [(24:1 (vol/vol)]. The solution was centrifuged for 5 minutes at maximum speed (16100 × g) and the supernatant discarded. The DNA precipitation step was carried out using polyethylene glycol (PEG) solution (30% (wt/vol) [PEG 6000, NaCl] and followed by 2 hours on ice incubation. The solution was centrifuged for 10 min at 4 °C and the pellet subsequently washed in ice-cold Dnase/Rnase free 70% ethanol, air-dried, and eluted in 30 µl 0.1% diethylpyrocarbonate water. Concentration and quality of DNA extracts were determined in duplicates using a Quant-iT™Pico Green® ds DNA assay Kit (Invitrogen, Carlsbad, USA) according to manufacturer's instructions. Measurements were performed with a SpectraMax Gemini EM Fluorescence Plate Reader Spectrometer (Molecular Devices, Sunnyvale, California, USA). To correct for background fluorescence, negative controls were added. Until further use, DNA extracts were stored at -80 °C.

2.7.2 *In silico* analyses of primer pair coverage

Analysis of the 16S rRNA (Figure 4) is a standard approach for cultivation-independent investigation of bacterial diversity within environmental samples, as it provides valuable phylogenetic information for the comparison of bacterial species (Janda & Abbott, 2007). So-called universal primers are usually designed to bind to the conserved regions of the 16S rRNA to amplify the variable regions, however, parts of these conserved regions of bacterial 16S rRNA are very similar to the conserved region of the 18S rRNA of plant mitochondria and the 16S rRNA of chloroplasts (Dorn-In et al., 2015). Thus, in environmental samples that are rich of plant DNA, non-targeted chloroplasts or mitochondria may be amplified, causing strongly biased results. To avoid the co-amplification of non-targeted chloroplast or plant mitochondrial DNA when studying bacterial endophytes in *Dactylis glomerata* L. an *in silico* analysis was carried out.

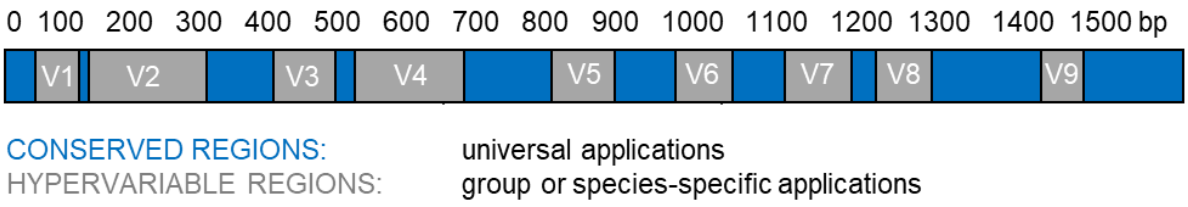

Figure 4: 16S rRNA

Conserved regions (blue) are targeted by universal primers to amplify bacterial diversity within environmental samples. Hypervariable regions (grey) are highly conserved regions that are used for group or species-specific amplification. Positions of hypervariable regions (V1 – V9) span nucleotides 69 – 99, 137 – 242, 433 – 497, 576 – 682, 822 – 879, 986 – 1043, 1117 – 1173, 1243 – 1294 and 1435 – 1465

for V1 through V9 respectively and are based on the *E. coli* system of nomenclature (Brosius et al., 1978).

2.7.2.1 Primer pair selection and *in silico* evaluation

Five sets of universal primers were used (Tables 2 and 3). Primer pairs were chosen according to amplicon length to perform sequencing of amplicons of the 16S rRNA gene on the Illumina MiSeq platform (Illumina Inc., USA) and coverage of variable regions to be able to identify bacterial species/genera in environmental samples by sequence analysis. The first and second primer pairs evaluated (No. 1 and 2; see Table 2), reportedly exclude chloroplast amplification in *Glycine max, Secale cereal, Triticum aestivum, Zea mays, Solanum tuberosum,* and *Lolium multiflorum.* In addition to this, primer pair No. 1 is also shown to exclude plant mitochondria of *Triticum aestivum* and *Lolium perenne* (Dorn-In et al., 2015). While primer pair No. 1, 335F/769R, is targeting the V3 – V4 region of the 16S rRNA gene and produces a fragment length of approximately 431 bp, primer pair No. 2 is targeting the V4 region and generates a fragment length of about 270 bp. The primer pairs No. 3 – 5 are reported to exhibit a high percentage of matches for a given taxonomic path, however, they are not specifically excluding chloroplast or mitochondrial DNA of plants.

The *in silico* evaluation of the primers was done with the TestPrime tool according to Quast et al. (2013), using the SILVA rRNA database (release SSURef 119 NR) containing 464 618 bacterial sequences. The SILVA SSURef 119 NR is using small subunit (SSU) sequences of the prokaryotic ribosomes that are longer than 1200 bp. An alignment quality value better than 50 (Pruesse et al., 2007) is required for sequences using SINA (Pruesse et al., 2012) alignment. Redundant sequences are removed by clustering with UCLUST (Edgar, 2010) with a 99% identity criterion. To simulate a realistic PCR behavior and mitigate unobserved sequence variation or other adverse conditions, “Maximum number of mismatches” was set to “1 mismatch” and “Length of 0-mismatch zone at 3’ end” set to “3 bases”. The resulting coverage of each primer pair, i.e. the percentage of matches for the taxonomic classification into bacteria or chloroplasts, respectively, is listed in Table 3.

Table 2: List of primers used for *in silico* analysis

Position on 16S rRNA	Primer name	Direction	Primer sequence [direction 5' – 3']	Primer reference
8 – 23	S-D-Bact-0008-a-S-16	Forward	AGAGTTTGATCMTGGC	Klindworth et al. 2013
335 – 352	335F	Forward	CADACTCCTACGGGAGGC	Dorn-In et al. 2015
341 – 357	S-D-Bact-0341-b-S-17	Forward	CCTACGGGNGGCWGCAG	Klindworth et al. 2013
519 – 536	Com1	Forward	CAGCAGCCGCGGTAATAC	Smalla et al. 2007
785 – 802	S-D-Bact-0785-a-S-18	Forward	GGMTTAGATACCCBDGTA	Baker & Cowan 2004
343 – 357	S-D-Bact-0343-a-A-15	Reverse	CTGCTGCCTYCCGTA	Klindworth et al. 2013
769 – 788	769R	Reverse	ATCCTGTTTGMTMCCCVCRC	Dorn-In et al. 2015
785 – 803	S-D-Bact-0785-a-A-21	Reverse	GACTACHVGGGTATCTAATCC	Klindworth et al. 2013
1100 – 1114	S-*-Univ-1100-a-A-15	Reverse	GGGTYKCGCTCGTTR	Klindworth et al. 2013

Table 3: List of primer pairs used for PCR

Primer pair No.	Forward Primer	Reverse primer	Ø length [bp]	Ø Tm [°C]	HV regions	Coverage Bacteria [%]	Coverage Chloroplast [%]
1	335F	769R	431	60	3 – 4	70	0
2	Com1	769R	270	60	4	88	85
3	S-D-Bact-0008-a-S-16	S-D-Bact-0343-a-A-15	350	50	1 – 2	87	80
4	S-D-Bact-0785-a-S-18	S-*-Univ-1100-a-A-15	330	47	5 – 6	83	90
5	S-D-Bact-0341-b-S-17	S-D-Bact-0785-a-A-21	465	54	3 – 4	90	84

2.7.2.2 Optimization of polymerase chain reaction (PCR) and primer selection

In order to construct and optimize an efficient amplification method, i.e. to minimize PCR amplification bias, but still retrieve qualitative PCR products and evaluate requirements for PCR conditions when analysing plant root samples, all primer pairs were tested using different thermal profiles (Table 4). The main parameters that were changed were the number of cycles and the elongation time. Each primer set was tested with 2 root DNA extracts and the respective rhizosphere extracts as a reference for bacterial amplification, resulting in a total of 24 reactions per primer set and sample extract. Each reaction mix contained 12.5 µl NEB Next High Fidelity Master Mix (Illumina Inc., USA), 0.5 µl of each primer (10 pmol/µl), 2.5 µl of 3% BSA, 100 to 200 ng of template DNA, and ad DEPC water to 25 µl. The following PCR conditions were used: initial denaturation step at 98 °C for 5 min, followed by the listed number of cycles for the respective thermal patterns of denaturation, annealing, and elongation thermal patterns seen in Table 4. The final elongation step was carried out at 72 °C for 5 minutes. For further analysis, PCR conditions were used where DNA amplicons showed the weakest band on the respective size (based on primer pair) on a 2% agarose gel to avoid overamplification.

Table 4: Thermal profiles of polymerase chain reaction optimization

Primer pair No.	Initial denaturation	Denaturation	Annealing	Elongation	No. Of cycles	Final elongation
1 / 2	98 °C, 5 min	98 °C, 10 s	60 °C, 30 s	72 °C, 20 s	20 / 25 / 28 / 30	72 °C, 5 min
				72 °C, 30 s		
				72 °C, 45 s		
3			50 °C, 30 s	72 °C, 20 s		
				72 °C, 30 s		
				72 °C, 45 s		
4			47 °C, 30 s	72 °C, 20 s		
				72 °C, 30 s		
				72 °C, 45 s		
5			53 °C, 30 s	72 °C, 20 s		
				72 °C, 30 s		
				72 °C, 45 s		

To evaluate the effect of primer bias on the abundance estimation of bacterial taxonomic groups, Illumina sequencing was performed. The PCR of the 16S rRNA region was conducted in triplicates with Illumina adapter sequences as described below (see chapter 2.7.3) using the evaluated conditions for the respective primer pair and sample extract respectively. Taxonomic classifications for each read were applied as described in chapter 2.8, using an abundance cut-off of 0.001%. The sequencing results revealed that primer pair No. 1, 335F/769R, was the only primer pair that amplified bacterial DNA almost exclusively in samples in which plant DNA prevailed (Table 3) and thus was used for further analysis in this study.

2.7.3 Library preparation and sequencing of amplicons of the 16S rRNA gene

Next generation sequencing was performed using the Illumina MiSeq platform (Illumina Inc., USA). Library preparation was accomplished according to the “16S Metagenomic Sequencing Library Preparation” protocol proposed by Illumina Inc., USA. Sequence files are deposited in the NCBI Sequence Read Archive under accession numbers SRP102620 and PRJNA380810.

Briefly, PCR of the 16S rRNA region was performed in triplicates using the primers 335F and 769R with Illumina adapter sequences. The reaction mix contained 25 µl mixture that was comprised of 12.5 µl NEB Next High Fidelity Master Mix (Illumina Inc., USA), 0.5 µl of each primer (10 pmol/µl), 2.5 µl of 3% BSA, 100 to 200 ng of template DNA, and ad DEPC water to 25 µl. PCR conditions included an initial denaturation step at 98 °C for 5 min, followed by 20 cycles for rhizosphere- and bulk soil samples and 28 cycles for roots samples, respectively, a denaturation step (98 °C; 10 s), an annealing step (60 °C; 30 s), and an elongation step (72 °C; 30 s). The final elongation step was carried out at 72 °C for 5 minutes. Negative control samples using DEPC water instead of template DNA were treated similarly. DNA amplicons were analyzed for the correct size using a 2% agarose gel, triplicates were pooled, and subsequently purified using the Agencourt®AMPure®XP (Beckman Coulter Company, USA) extraction kit according to manufacturer’s instructions. However, the ratio of AMPure XP to PCR reaction was changed to 0.6 instead of 1. Amplicon sizes and presence of primer-dimers were checked using a Bioanalyzer 2100 (Agilent Technologies, USA), the DNA 7500 kit (Agilent Technologies, USA), and quantified using the Quant-iT PicoGreen kit (Life Technologies, USA). Afterwards, indexing PCR was performed using 12.5 µl NEB Next High Fidelity Master Mix, 10 ng DNA of the previous PCR products, and 10 pmol of each primer containing adapter overhangs. For each indexing PCR, annealing temperature was reduced to 55 °C and the number of cycles was reduced to eight cycles. Purified PCR products were pooled in equimolar ratios to a final concentration of 4 nM and sequenced using the MiSeq Reagent kit v3 (600 cycles) (Illumina Inc., USA) for paired end sequencing.

2.8 Filtering and taxonomic classification of sequencing data

Sequences were analyzed using QIIME suite (Quantitative Insights into Microbial Ecology) software package version 1.9.1 (Caporaso et al., 2010) and default parameters. Raw sequencing data was filtered by removing adapter sequences and trimming terminal nucleotides with a minimum read length of 50 bp and a Phred quality score lower than 15 using AdapterRemoval (Schubert et al., 2016). PhiX decontamination was performed with DeconSeq (Schmieder & Edwards, 2011). Afterwards, reads were merged, filtered by amplicon size (400 – 480 bp), and quality (Phred quality score >2). Subsequently, sequences were clustered into operational taxonomic units (OTUs) with an open reference strategy at a 97% sequence similarity using the GreenGenes 16S rRNA as the reference database (13_5 release; DeSantis et al., 2006). Clustered reads were taxonomically classified against the RDP classifier (Wang et al., 2007), which is retrained on the GreenGenes 16S rRNA reference database. Remaining chloroplast assigned sequences were removed and the output was filtered with an abundance cut-off of 0.005% (**Manuscript 1**) or 0.001% (**Manuscript 2**), respectively. Subsequently, the data was rarefied to the lowest obtained read number to make results comparable.

2.9 Statistical analysis and data visualization

Prior to statistical analysis and data visualization, a core set of diversity analyses were performed using QIIME implemented work packages. These include the pre-processing into relative abundances of each OTU per sample and the computation of α- and ß-diversity. The within sample diversity (α-diversity) was based on chao1 richness, Shannon's diversity (**Manuscript 2**) and Faith's phylogenetic diversity (**Manuscript 1**). Measures were computed using the QIIME script "alpha_diversity.py". Weighted and unweighted Unifrac distances as well as Bray-Curtis dissimilarity (normalized Manhattan distance) were used for ß-diversity measures and computed using the QIIME script "beta_diversity.py".

The statistical analysis and data visualizations were performed using the R environment (version 3.2.1; R Core Team, 2014). Significant differences in α-diversity were determined using unpaired *t*-tests (**Manuscript 2**), whereby p values less than 0.05 were considered statistically significant and calculated per compartment, LUI, and sampling season/plant developmental stage (**Manuscript 2**). The graphs were made by means of the functions ggplot in the packages "ggplot2" (Wickham, 2009), "gplots" (Warnes et al., 2016), and "sciplot" (Morales, 2011), respectively. Dissimilarity between samples was examined by constrained principal coordinates analysis (PCoA) and based on Unifrac and Bray-Curtis distances. These were created using the pcoa function within the "ape" and "vegan" packages (Paradis et al., 2004). Datasets were then analyzed with permutational multivariate analysis of variance test (ADONIS) using

the function adonis within the “vegan” package. Pseudo-*F* ratios below 0.05 were considered statistically significant. Significances were determined between LUIs in the different compartments (**Manuscript 1**) and high versus low LUI in different compartments and plant developmental stage or significant differences between high versus low LUI within each plant developmental stage, respectively (**Manuscript 2**). The correlation of environmental factors (**Manuscript 1**) was tested by canonical analyses of principal coordinates using the measures for soil characteristics as constraints. The canonical correlation analysis was calculated by means of the functions plot_cap and capscale implemented in the “vegan” package (Paradis et al., 2004). Significances were calculated using the ANOVA-like permutation analysis function anova.cca included in the “ape” and “vegan” packages (Paradis et al., 2004). P values below 0.05 were considered statistically significant. Ternary plots (**Manuscript 1**) were visualized by means of the function ternary plot within the “vcd” package (Friendly, 2017), which were computed for high versus low LUI. The computation of a bacterial core taxa was performed applying the QIIME script “compute_core_microbiome.py” and was based on the total relative abundance of bacterial OTUs. Briefly, core OTUs were defined as OTUs that are present in at least 90% of the samples grouped into high versus low LUI in each compartment for every plant developmental stage. The calculation and visualization were performed by applying VENN diagrams (http://bioinformatics.psb.ugent.be/webtools/Venn/).

3 Manuscript overview

This thesis is based on the following publications:

- Estendorfer, J., Stempfhuber, B., Haury, P., Vestergaard, G., Rillig, M. C., Joshi, J., Schröder, P., & Schloter, M. (2017). The Influence of Land Use Intensity on the Plant-Associated Microbiome of *Dactylis glomerata* L. *Frontiers in Plant Science, 8,* 930. https://doi.org/10.3389/fpls.2017.00930
  (Manuscript 1, first author, published)

- Estendorfer, J., Stempfhuber, B., Vestergaard, G., Schulz, S., Rillig, M. C., Joshi, J., Schröder, P., & Schloter, M. (2020). Definition of core bacterial taxa in different root compartments of *Dactylis glomerata*, grown in soil under different levels of land use intensity. *MDPI - Diversity*, *12*(10), 392. https://doi.org/10.3390/d12100392
  (Manuscript 2, first author, published)

Manuscript 1

The Influence of Land Use Intensity on the Plant-Associated Microbiome of *Dactylis glomerata* L.

Jennifer Estendorfer, Barbara Stempfhuber, Paula Haury, Gisle Vestergaard, Matthias C. Rillig, Jasmin Joshi, Peter Schröder and Michael Schloter

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In this manuscript, we investigated the influence of different land use intensities (LUI) on the root-associated microbiome of *Dactylis glomerata* L. as well as the driving factors for bacterial composition within plants in the reproductive stage. For this purpose, *Dactylis glomerata* L. samples that were in its reproductive phase were collected in early summer 2015. The experimental sites ranged from natural grassland to intensively managed meadows. Using sequencing of amplicons of the 16S rRNA gene, bacteria in the plant endosphere, rhizosphere, and bulk soil were identified. The findings suggest that roots harbor a distinctive bacterial community compared to the rhizosphere and bulk soil, resulting in a clearly distinguishable pattern of bacterial communities under different LUI compared to the rhizosphere and bulk soil. While dominant families in the root endosphere were classified into *Pseudomonadaceae*, *Enterobacteriaceae,* and *Comamonadaceae*, irrespective of LUI, the most abundant families strongly differed in rhizosphere and bulk soil on different LUIs. Furthermore, the effects of LUI are more pronounced in rhizosphere and bulk soil compared to the root endosphere. Overall, a change in community structure at the plant-soil interface was observed as the number of closely related bacterial groups between endosphere, rhizosphere, and bulk soil increased with decreasing land use intensity. Hence, results suggest a stronger interaction of the plant with the surrounding soil at low land use intensity by selectively attracting bacteria from the surrounding soil. In addition, the amount and quality of available soil nitrogen was identified as a major factor for changes in microbiome structure in all three compartments.

JE designed the experiment, carried out the field work, laboratory experiments, data analysis, and wrote the manuscript. MS, BS, and PS contributed to the design of the experiment. PH contributed to the field work and the laboratory experiments. GV provided the pipelines for sequencing data analysis and advised data analysis. BS, PS, GV, JJ, MR, and MS advised the studies and critically revised the manuscript.

Manuscript 2

Definition of Core Bacterial Taxa in Different Root Compartments of *Dactylis glomerata*, Grown in Soil under Different Levels of Land Use Intensity

Jennifer Estendorfer, Barbara Stempfhuber, Gisle Vestergaard, Stefanie Schulz, Matthias C. Rillig, Jasmin Joshi, Peter Schröder and Michael Schloter

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This manuscript evaluates core bacterial assemblages in the rhizosphere and endosphere of *Dactylis glomerata* L., assesses seasonal variations, and the impact of contrasting LUIs on core bacterial taxa to be able to predict community responses to environmental changes. *Dactylis glomerata* L. samples were collected from grassland sites with contrasting land use intensities but comparable soil properties at three different timepoints. Bacterial community structure was identified using sequencing of amplicons of the 16S rRNA gene. A distinct composition of plant-associated core bacterial communities independent of land use intensity was identified in all compartments. Due to the frequent occurrence of plant-promoting capabilities within the genera found in the plant-associated core bacterial communities, this study suggests a “healthy” plant-associated bacterial community. Low land use intensity was associated with an increased variable component of the plant-associated microbiome, as indicated by the fluctuation of taxa over different sampling times. Comparing samples from high and low land use intensity plots, we found greater compositional variation in samples obtained from low land use plots, suggesting a more selective recruitment of bacteria with traits important for various stages of plant development.

JE designed the experiment, carried out the field work, laboratory experiments, data analysis, and wrote the manuscript. MS, BS, and PS contributed to the design of the experiment. GV provided the pipelines for sequencing data analysis and advised data analysis. BS, PS, GV, SS, JJ, MR, and MS advised the studies and critically revised the manuscript.

4 Discussion

Plant-associated bacteria play an important role for plant fitness and together with its host, are considered as a functional unit, the plant holobiont (Berg et al., 2020). In turn, associated bacteria are essential for the health and functioning of ecosystem services. With land use intensification being the major anthropogenic cause for the decrease of diversity in agricultural systems, and thus threatening the amount of ecosystem services provided by agricultural habitats, it is crucial to gain insight into the diversity and composition of bacterial assemblages within different plant compartments subjected to different land use intensities in field-realistic conditions to be able to mitigate further loss of biodiversity. Comprehensive studies on response patterns of plant-associated bacteria in “real world” land management systems, where several environmental and anthropogenic influence factors may cause a shift in composition and diversity of bacteria are missing (Berg et al., 2020). Furthermore, studies that investigate the impact of a continuous land use intensity measure on bulk soil, the rhizosphere, and the plant-associated microorganisms are scarce. Yet, the mechanisms governing the structure of plant-associated microbial communities, which influence their function, are still largely unknown.

Hence, one of the central aims of this study was to identify plant-associated bacteria within different compartments and plant developmental stages of *Dactylis glomerata* L. with the focus on the response patterns of bacterial communities towards the anthropogenic factor of land use intensity. The experiments were conducted within the framework of the BEO, which provided a field-realistic environment and the application of a continuous measure of LUI.

4.1 Diversity patterns of plant-associated bacteria in response to LUI

In this respect, the first hypothesis (**i**) postulated that the impact of LUI on bacterial diversity and composition will be most pronounced within the rhizosphere and bulk soil compared to the root endosphere. Conversely, root endophytic communities are less affected by LUI as they are primarily mediated by the host plant. As a result, the composition of bacteria within the endosphere will strongly differ to the rhizosphere and bulk soil and the structure of bacterial assemblages will be less diverse under high compared to low land use intensities in all compartments.

As expected, the α-diversity and community structure (as represented by chao1 and Shannon diversity index) of bacteria strongly differed between rhizosphere, bulk soil, and endosphere, with higher diversity in the rhizosphere and bulk soil compared to the root endosphere at all sampling dates. The diversity within the rhizosphere and bulk soil was comparable **(Manuscript 1 & 2)**. These results were expected due to no physical delineation of the bulk soil and

the rhizosphere. The roots, however, are a physically separated compartment from its surrounding soil, thus hampering colonization not only through spatial differentiation, but through selective attraction and only some bacteria being able to enter the plant roots (Hardoim et al., 2015). Successful endophyte colonization has been shown to involve compatible plant-host interactions. Various studies have recognized the chemotactic response of endophytes to plant root exudates (Bergelson et al., 2019; Brader et al., 2017; Rosenblueth & Martínez-Romero, 2006). Upon bacteria entering the host plant, the endophyte is recognized by the plant and the crosstalk of signalling molecules is initiated, therefore leading to selective filtering and limiting bacterial colonization of the host plant and a decrease in biodiversity from rhizosphere and bulk soil to root endosphere. This indicates an increasing influence of host-specific factors from bulk soil to rhizosphere to root endosphere and the plant compartment being a major selective force for shaping the diversity and composition of plant-associated bacterial communities. When further separating the diversity measures into high versus low LUI within each plant developmental stage, a significant influence of LUI on α-diversity was detected for bulk soil in early summer. In contrast to our expectation though, diversity measures were observed to be increased under high LUI compared to low LUI. These differences most likely arose from the variable land management applications on the sampling sides, which leads to a fluctuating resource availability and in turn to a more variable microbial community composition and an uneven distribution of certain bacterial taxa. Similar results were observed by Garbeva et al. (2008), who suggested that the composition of microbial communities is highly variable due to the strong impact of the various stimulatory and destimulatory effects of management applications that promote bacterial proliferation. Impacts on the so-called *r*-strategists, which are bacteria that are characterized by high growth rates under high nutrient supply and fluctuating nutrient availability conditions (Garbeva et al., 2008), are most pronounced. This in turn may lead to a transient and discontinuous bacterial growth pattern and a high variability on intensively managed sites. In contrast, bacterial diversity in undisturbed environments with low human impact are considered to be more stable in terms of nutrient availability and input and thus may be virtually refractory to change due to being adapted to the highly competitive environment. Low resource availability on low LUI plots may promote so-called *K*-strategists, bacteria that are slow-growers, but have high resource-acquiring affinities and high yields (Pettersen et al., 2021). Hence, low LUI plots are considered more stable environments, which leads to a more uniform colonization and less overall diversity compared to high LUI. Although the studied gradient of land use intensity ranged from unfertilized meadows and pastures to highly fertilized meadows and mown pastures, an impact neither was detected on rhizosphere and endosphere inhabiting communities for all plant developmental stages nor in soil communities in May or October **(Manuscript 2)**. These findings may be explained due to bacteria that may adopt different strategies during different times of the year based on local conditions,

which can result in co-occurrence or co-exclusion of certain bacterial taxa, which in turn result in no significant difference in α-diversity measures. Furthermore, some bacteria have the capability to switch between strategies to ensure the survival under a broad range of environmental conditions and thus cannot always be categorized neatly into *r*- versus *K*-strategists (Barberán et al., 2012; Pettersen et al., 2021; Song et al., 2017). These results correspond to results found in other studies that could show that microbial communities are more strongly influenced by plant species rather than soil characteristics, as plant roots exert a species-specific variety of compounds into its surrounding soil including ethylene, sugars, proteins, phenolics, amino acids, organic acids, vitamins, polysaccharides, enzymes, and other secondary metabolites (Chagas et al., 2017; Garbeva et al., 2008; Jones et al., 2009; Praeg et al., 2019). These released compounds create unique environments that, together with climatic conditions and edaphic factors, shape the bacterial communities within the rhizosphere and creates a desirable niche for microbial communities to proliferate (Trivedi et al., 2020). As the commonly used measures for taxonomic richness usually count the numbers of species within the respective sample, phylogenetic diversity (PD), a measurement of branch length within a phylogenetic tree, was applied to provide an evolutionary measure. PD provided further insight into how much phylogeny and evolutionary history can be found on high versus low land use intensity plots (Miller et al., 2018). In addition to the influence on soil communities in early summer, phylogenetic diversity revealed a significant impact of LUI on the root endosphere inhabiting bacterial communities in early summer, with low LUI exhibiting higher diversity. However, during the other plant developmental stages phylogenetic diversity was not significantly impacted by LUI **(unpublished data)**. These results indicate the co-existence of closely related bacteria in the plant endosphere during the plants reproductive stage, which is probably owed to the greater impact of the host plant on the assembly of the inhabiting microbiome due to specific metabolic demands during plant reproduction.

Analysis of β-diversity (between sample diversity) was based on the computation of Unifrac metrics. Unifrac metrics incorporate information on the relative relatedness of community members based on phylogenetic distances between observed taxa to exploit different degrees of similarity between sequences (Lozupone & Knight, 2005). Weighted Unifrac are the quantitative measure and use the abundance of each taxon. Thus, the quantitative measure of β-diversity is used to detect changes in the number of sequences of each lineage and changes in the number of taxa present. The unweighted Unifrac is a qualitative measure, which only use the presence or absence of data as duplicate sequences do not contribute additional branch length to the tree (Lozupone et al., 2007). Analysis of β-diversity in the root endosphere revealed that a significant impact of LUI could only be detected during the reproductive stage (June) of *Dactylis glomerata* L. for the unweighted Unifrac metric, but neither during other stages of the plant nor for the weighted measures **(Manuscript 1 & 2)**. This indicates that LUI

only has an impact on the presence and absence of certain bacterial taxa in the root endosphere during the plant's reproduction, but does not impact the relative taxon abundance, which in turn primarily differentiates taxa that can live under low nutrient supply. Consistent with α-diversity measures for the root endosphere, these results indicate a specialized community composition under low LUI during seed production that is formed by the presence of *K*-strategists or taxa that are specialized to metabolize limiting resources. In contrast, high nutrient availability that is introduced through high land use management does not cause a particular group of taxa to thrive in the plant endosphere, suggesting bacterial composition being highly regulated by the plant. Due to seed production during the reproductive stage, which imposes high metabolic cost on the plant through the synthesis of storage products (including proteins, starch, and lipids), the uptake of minerals, and the shift of nutrients from the site of synthesis to the seed assimilation is required (Munier-Jolain & Salon, 2005). Nitrogen is an essential nutrient for the growth of plants and their associated microorganisms, the conversion of nitrogen into organic and inorganic forms is a key process for maintaining productivity and relevant ecological functions and services (Urakawa et al., 2014). Thus, under low LUI, where nutrient deficiency is seen through reduced amounts of plant-available nitrogen, it is important to establish a specific microbiome, which has the ability to metabolize plant-available nitrogen either through mineralization of dead biomass or nitrogen fixation to achieve optimal performance of the plant (Gao et al., 2021; Pankievicz et al., 2019). Under high LUI, specific attraction is less important due to nutrient input through fertilization, which might explain the change in β-diversity in June between sites with different land use intensities. While the number of bacterial taxa observed in high versus low LUI did not differ significantly (α-diversity) within the rhizosphere, rare and transient taxa dominated qualitative measures, caused by a significant influence of LUI that was observed for the unweighted Unifrac metric during all plant developmental stages. Abundant species in the rhizosphere, however, were not impacted by LUI (weighted Unifrac), hence, it was concluded that high abundant taxa are similar between high and low LUI, and significance was attributed to the presence or absence of low abundant taxa **(Manuscript 2)**. These results suggest that low abundance bacteria dominate plant species-specific responses to LUI. Most research, however, has focused on frequently occurring and dominant community members rather than on less dominant taxa despite the increasing attention for rare bacterial taxa (Aanderud et al., 2015; Jousset et al., 2017). Conversely, the abundance and metabolic activity are not intimately related (Hunt et al., 2013). Rare species can have a significant impact on biogeochemical cycles and can be essential for the functional potential of the microbiome, thereby driving ecosystem processes (Jousset et al., 2017). Previous studies have demonstrated that low abundance of specific taxa might be driven by changes in abiotic conditions, such as LUI, and are more vulnerable to environmental fluctuations and more likely to become extinct (Gaston, 2008). Land use intensification can therefore

have a profound impact on the rare biosphere and associated ecosystem functions (Rodrigues et al., 2013). Jousset et al. (2017) suggested that rare microbes are likely to be overlooked keystone species in regulating host-associated environments in terrestrial habitats. Hence, it is of crucial importance to identify rare species to be able to conduct further investigations on changes in low abundant microbial species and their metabolic activity in response to anthropogenic factors.

In accordance with my expectations of the first hypothesis (**i**), the influence of LUI increased in bulk soil compared to the other compartments as significant differences were observed in both weighted and unweighted metrics throughout all plant developmental stages between high and low LUI. The only exception for the bulk soil was the weighted metric in October, which reflected the senescence stage of the plant. These results suggest that in addition to the presence and absence of certain taxa, species abundance in bulk soil is affected by LUI during the sampling dates of May and June, i.e. when the plants were in the vegetative and reproductive stage. Furthermore, the degree and direction towards shifts in the abundance as well as the presence/absence of single OTUs increased from root endosphere to rhizosphere and bulk soil in response to LUI and is dependent on the plant developmental stage, which is reflected by the significance level and the variance for both metrics. This underlines that the influence of LUI is less pronounced in the root endosphere and that the plant is the major selective force in shaping the diversity and composition of associated communities with an increasing importance from soil to rhizosphere to the plant root endosphere.

Interestingly, these results are only partly consistent with other studies on belowground microbial communities subjected to different levels of LUI. Previous studies that were conducted at the same grassland sites did not find a significant effect of different management regimes on soil bacterial diversity and composition (Herold et al., 2014; Kaiser et al., 2016). Both studies, however, did not investigate differences within several sampling dates, but were rather performed in early Spring, whereas samples for this thesis were collected throughout different time periods. Accordingly, resource availability is affected by seasonal fluctuations in soil and plant-associated microbial community composition, which are influenced by C allocation, nutrient uptake, and plant litter, as well as the specific timeframes of land management applications (Koranda et al., 2013). These changes in resource availability and edaphic parameters are supported by the measurements of soil parameters as well as the significant differences in β-diversity between the sampling seasons **(Manuscript 2)** within this thesis. Furthermore, both studies did not use the multivariate Unifrac technique for comparing microbial communities in a phylogenetic context, which might have led to these different outcomes. In contrast to studies conducted at sites of the BEO, other investigations on a management intensity gradient found that soil bacterial diversity was the highest on moderately managed soil (Tardy et al., 2015).

Results were attributed to the dominance of particularly competitive species through competitive exclusion in highly stressed environments versus the dominance of particularly adapted species through selection in unstressed environments. As a result, moderate stress as represented within moderately managed soil environments may increase apparent diversity by lowering the likelihood of competitive exclusion and selection. These studies, however, are difficult to compare as they were not carried out at the same sites and the definition of land use and management, and the period of impact vary considerably between the studies. As the land use intensity is considered a multifactorial influence, the study of Tardy et al. (2015) does not reflect LUI as a continuous measure that constitutes different management components.

4.2 Composition of plant-associated bacteria during different plant developmental stages

The second hypothesis (**ii**) of this thesis postulated that the bacterial community composition within the root endosphere is distinctive in contrast to the rhizosphere and bulk soil, as the plant will selectively shape its associated bacterial microbiome throughout the different developmental stages and only few bacterial taxa being able to enter the plant inner tissue. In contrast, the bacterial community composition in the surrounding rhizosphere and bulk soil will be comparable as there is no physical obstacle to overcome.

To verify the hypothesis (**ii**), 16S rRNA amplicons were taxonomically classified. Classification revealed Pseudomonadota (formerly Proteobacteria) as the dominating bacterial phylum in all compartments, followed by Bacterioidota (formerly Bacterioidetes), Actinobacteriota (formerly Actinobacteria), Acidobacteriota (formerly Acidobacteria), and Bacillota (formerly Firmicutes) in all compartments and stages of *Dactylis glomerata* L. The structural composition, however, differed between all three compartments and stages **(Manuscript 1 & 2)**. While the root compartment primarily consisted of Pseudomonadota (roughly 90%), the phyla Bacterioidota, Actinobacteriota, and Acidobacteria showed a higher abundance in the rhizosphere and bulk soil compartments compared to the endosphere. Conversely, despite Pseudomonadota being the prevailing phylum in all compartments, the abundance increased with proximity to the plant root showing the least abundance in bulk soil compared to the other compartments. Previous analyses on bacterial communities in soil samples collected at the same sites are partly in line with these results. While Pseudomonatoda were found to be the most abundant phylum, Acidobacteriota and Actinobacteriota are claimed to be highly abundant in the soil in the “Schwäbische Alb” (Foesel et al., 2014; Kaiser et al., 2016). However, all analyzed samples were taken during a single sampling campaign and included all 50 grassland plots within the exploratory of the “Schwäbische Alb” (Kaiser et al. 2016; Foesel et al. 2014). Hence, seasonal variations as well as land use management are not reflected in the abundance measurements

in a similar manner as in the present study. Moreover, in addition to LUI, edaphic factors and soil type vary throughout the different plots. Previous studies have shown that soil properties are a major driver of soil microbial diversity and composition (Bastida et al., 2021; de Gannes et al., 2015; Kaiser et al., 2016). This in turn may have a strong impact on overall soil bacterial composition within the exploratory and lead to skewed results. To avoid generalized results, plots with comparable edaphic factors were selected for the present study and therefore reflect soil bacteria more accurately with respect to soil type and seasonal variations.

In consistence with our expectations, the root endosphere harbors a distinct community composition when comparing at lower taxonomic ranks and was largely dominated by *Pseudomonadaceae* throughout all plant developmental stages and independent of LUI. Other abundant taxa were assigned to *Enterobacteriaceae, Comamonadaceae, Rhizobiaceae, Xanthomonadaceae, and Bradyrhizobiaceae*. However, abundance was influenced by the plant developmental stage **(Manuscript 2)**. Seasonal changes in bacterial community patterns reflect the respective growth phase, changing physiology, and external temperatures changes (Jansson & Douglas, 2007; Shen & Fulthorpe, 2015). These affect the concentrations of soluble sugars, proteins, amino acids, organic acids, and other nutrients within the plant and in turn influences the establishment of associated bacterial communities in the root endosphere (Li et al., 2004; Renaut et al., 2005; Shen & Fulthorpe, 2015). Despite *Pseudomonadaceae* were the most abundant family throughout all plant developmental stages, they were found to be enriched in the vegetative stage (May) compared to the reproductive (June) and senescence (October) plant stage, *Comamonadaceae* were found in slightly higher abundances during the vegetative (May) and flowering stage (June). The other abundant families did not show remarkable differences across different growth stages. *Pseudomonadaceae* having the highest abundance in the root endosphere is in consistence with other studies that investigated plant colonizing bacteria in perennial grasses (Wemheuer et al., 2017). Species within this family, which were mainly assigned to *Pseudomonas* spp., are described as being among the most efficient plant growth-promoting bacteria due to their wide range of plant growth-promoting traits (Trivedi et al., 2020). These include the production of cytokinins and various plant hormones, siderophore production, phosphorus and potassium solubilization and mineralization, sulfur oxidation, cellulose synthesis, and have even been observed to supress disease in several plant-pathogen systems due to their biocontrol capabilities (Mitter et al., 2021; Roquigny et al., 2017). In addition to this, several members of *Enterobacteriaceae, Comamonadaceae, Rhizobiaceae, Xanthomonadaceae, and Bradyrhizobiaceae* are frequently reported to be found inside roots and to possess plant growth-promoting characteristics (Bulgarelli et al., 2013; Bulgarelli et al., 2015; de Santi Ferrara et al., 2012; Erlacher et al., 2015; Mitter et al., 2021; Ren et al., 2015). Nitrogen-fixing symbiosis in root nodules of legumes is the best studied symbiotic association of *Rhizobiaceae* and *Bradyrhizobiaceae* and is of major importance for

nitrogen input in both agricultural and natural ecosystems (De La Pena et al. 2018). Lineages within these taxa were described as diazotrophic bacteria, i.e. able to fix atmospheric nitrogen by expressing *nod* and *nif* gene clusters for nodulation and nitrogen fixation in legumes, thereby increasing plant performance under limited nitrogen availability (Erlacher et al., 2015; Wang et al., 2012). However, numerous studies indicate that they are not limited to leguminous plants but are also ubiquitously found within various endophytic compartments on non-leguminous plants, albeit without nodule formation (Erlacher et al., 2015; Rouws et al., 2014). The high abundance during all plant developmental stages, that is irrespective of LUI, indicates the importance of biological nitrogen fixation in *Dactylis glomerata* L. Added to this, the members of all families found in high abundance are associated with numerous traits that enhance plant nutrition through either mobilizing or increasing nutrient availability in soils. In addition to nitrogen fixing abilities these include the production of phytohormones, active secondary metabolites and antimicrobial compounds, the oxidation of sulfur, the chelation and solubilization of micronutrients, and the metabolism of vitamins and co-factors (Eid et al., 2021; Lee et al., 2019; Mącik et al., 2020). Due to their high efficiency regarding plant growth-promotion, some strains within the highest abundant families found have even been proposed as environmentally friendly biofertilizers for sustainable agriculture, further emphasizing their importance in the present study (Lally et al., 2017; Mitter et al., 2021). In contrast to the findings in the current study, Wemheuer et al. (2017), who investigated bacterial endophyte communities of different grass species and their response to different management practices, found *Massilia,* a genus within *Oxalobacteraceae*, to be the most abundant root endophyte in *Dactylis glomerata*. However, despite the family *Oxalobacteraceae* being found in all samples in the current study, the genus *Massilia* was only found in the root endosphere in October on both LUIs **(Manuscript 1 & 2).** Interestingly, Wemheuer et al. (2017) took their samples in Autumn, when the plants already reached their senescence stage, which concurs with our findings. Various studies have shown that sampling time and developmental stage strongly influence the phenotype of the plant also reflecting different metabolic demand of the plant, in turn altering associated microbial community patterns (Bevivino et al., 2014; Chaparro et al., 2014; Dreccer et al., 2013). These results further highlight the selective recruitment of the plants associated microbiome throughout the different plant developmental stages in response to the current metabolic requirements.

In contrast to the distinctive composition of bacterial communities within the root endosphere, the abundance and composition of bacteria residing in the rhizosphere and bulk soil were comparable. Despite *Pseudomonadaceae* being highly abundant in the roots, they were only found in very low abundances in both the rhizosphere and bulk soil. No specific taxon was as dominantly found as *Pseudomonadaceae* or *Enterobacteriaceae* within the roots, which leads to a more uniform distribution of taxa with respect to abundances in the rhizosphere and bulk soil.

The microbiome found in the bulk soil was found to be very similar to the rhizosphere, which is attributed to the close proximity to other plant roots in grassland soils that influence their surrounding environment. As there is no spatial delineation, the sampled “bulk soil” might be the rhizosphere of neighboring plant roots. Thus, the most commonly found families in both compartments were *Comamomadaceae, Sinobacteraceae, Hyphomicrobiaceae, Xanthomonadaceae, Chitinophagaceae, Bradyrhizobiaceae, Sphingomonadaceae,* and not further classified taxa within the orders *Myxococcales, SC-I-85,* and *Ellin6068*. The more evenly distributed abundance patterns, however, show a clear difference to the diversity pattern of the root endosphere. However, the overlap of taxa found in the root endosphere compared to rhizosphere/bulk soil hints at the “rhizosphere effect”, i.e. the structural and phylogenetic diversification of bacteria of the microhabitat endosphere is composed of soil biota surrounding the roots.

Hence, these results provide a first indication of the plant’s selective recruitment of bacterial taxa from its surrounding soil throughout the different plant developmental stages, which is in accordance with previous studies and in line with the expectations of the second hypothesis (**ii**) that the root endosphere of *Dactylis glomerata* L. has a distinctive community composition through selective attraction. In contrast, the rhizosphere and bulk soil were comparable and more diverse.

4.3 Land use intensity as a major driver of shifts in community composition

The aim of the third hypothesis (**iii**) is to disentangle and determine the influence of LUI and the putative feedback loop on plant-associated communities at different stages of plant development to verify that LUI is a major driver of changes in community composition through shaping soil properties and thus changing the nutrient availability. This section is divided into two parts. The first part discusses the significant shifts in bacterial communities in response to LUI throughout the different plant developmental stages whereas the second part analyzes the major influence factors to confirm that LUI has the highest impact on communities within the root endosphere.

4.3.1 The impact of LUI on plant-associated bacteria during different plant developmental stages

In order to disentangle and determine the impact of LUI and thus verify the feedback loop on plant-associated communities, bacterial abundances were compared in the high and low LUI samples at each stage of plant development. No significant impact of LUI was detected in each

stage on prevailing bacterial taxa in the root endosphere. Significant differences of LUI with respect to the abundance of assigned families were mainly detected in a few low abundant taxa, which is consistent with our findings within the unweighted metrics of the β-diversity analyses. Out of 130 classifications of OTUs into bacterial families in the root endosphere, only five families were significantly impacted by LUI in May, five in June and two in October **(Manuscript 1, unpublished data)**, albeit all of them in very low abundances (each less than 1% across all samples). During the plant's vegetative stage (May), these were assigned to *Patulibacteraceae*, *Sphingobacteriaceae, Rhodobacteraceae, Acetobacteraceae,* and not further assigned members of *IS-44* **(unpublished data)** and were found in lower abundances on high LUI plots. During the reproductive stage (June) OTUs significantly impacted by LUI were assigned to *Bryobacteraceae*, *Cytophagaceae,* and several unassigned members of *Rhizobiales* and were found to be more abundant under low LUI versus high LUI. In contrast, *Turicibacteraceae* were more prominent under high LUI **(Manuscript 1)**. *Sphingomonadaceae* and *Erythrobacteraceae* were the only families that were impacted by LUI during the plant's senescence stage (October) and showed a higher abundance on low LUI plots versus high LUI plots **(unpublished data)**.

4.3.1.1 The vegetative stage

Members of *Patulibacteraceae* were previously extracted from urban tree species (Shen & Fulthorpe, 2015), Miscanthus seeds (Cope-Selby et al., 2017), and in the rhizosphere of *Parastrephia quadrangularis* (Zhang et al., 2022), but are not further described as plant-promoting taxa. Jin et al. (2016) found that isolated members of *Patulibacteraceae* exhibit oxidase and catalase activities. Some strains show enhanced growth on agar medium that is supplemented with superoxide dismutase, which is known to catalyze the dismutation of superoxide to hydrogen peroxide and provides defence against oxidative stress (Leonowicz et al., 2018). Oxidative stress during the initial stages of the plant emerging can be caused in response to insufficient usage of excitation energy for photosynthetic photochemistry and lead to plant growth retardation (Leonowicz et al., 2018; Xie et al., 2019). In addition, mineral-deficiency, nitrogen in particular, is known to induce oxidative stress responses in plants (Matić et al., 2021) and exhibits the lowest amount in soils sampled in May on low LUI plots, which might cause the higher abundance of *Patulibacteraceae* in low LUI samples during the plant's vegetative stage (May). However, their role in promoting plant growth has yet to be confirmed.

The *Sphingobacteriaceae* that were found impacted by LUI in May could be further classified to the genus of *Pedobacter*. The species of this genus are commonly described as plant growth-promoting endophytes in oilseed rape and some cereal crops and are known for their ability to produce indole-3-acetic acid (IAA) (Lay et al., 2018; Yuan et al., 2011). IAA is one of

the most important phytohormones of the auxin class and plays a crucial role in plant growth and development by regulating key processes such as tissue differentiation, fertility, cell division, alignment, enlargement, and increase in the number of lateral roots and root hairs involved in nutrient uptake (Finet & Jaillais, 2012; Keswani et al., 2020; Zhao, 2010). Due to their importance for plant performance, recent studies are investigating auxins of microbial origin for use in agriculture (Keswani et al., 2020; Tan et al., 2021). This could be the reason for the significant increase of *Pedobacter* on plots with low LUI in the vegetative stage (May) to support plant development and nutrient uptake.

Among other not further classified *Rhodobacteraceae,* genera found were identified as *Amaricoccus, Paracoccus,* and *Rhodobacter*. All three genera are frequently reported to be extracted from plant endosphere and rhizosphere (Deng et al., 2011; Eid et al., 2021; Kobayashi & Aoyagi, 2019; Liu et al., 2019; Tangapo et al., 2018). Species within *Rhodobacter* have been demonstrated to fix nitrogen in resource limited environments by either a molybdenum-dependent or a molybdenum-free iron-only nitrogenase and thus actively enhancing nutrient bioavailability in soil and plant biomass production (Hoffmann et al., 2014; Mburu et al., 2021). Furthermore, *Rhodobacter* spp. are frequently reported as plant growth-promoting bacteria as they are able to mitigate environmental stress by producing IAA in early plant developmental stages and significantly improving root length, shoot length, as well as plant biomass (Kang et al., 2020; Kang et al. 2021). In addition, some *Paracoccus* spp. were found to promote plant growth in the endosphere through siderophore iron complex production to contribute to iron uptake in plants (Deng et al., 2011; Zhang et al., 2019) and are found within the rhizosphere and endosphere of numerous plants (Campisano et al., 2014; Kämpfer et al., 2012; Lin et al., 2017). Furthermore, they have been found to be significantly correlated to organic pest management in grapevine (Campisano et al., 2014). In contrast, *Amaricoccus* have not been further described as plant growth-promoting taxa despite them being frequently found as a plant-associated genus in tomato (Grunert et al., 2020), banana (Liu et al., 2019), potato (Rosenzweig et al., 2012), or sweet potato (Tangapo et al., 2018). Conversely, *Amaricoccus* is associated with waste-water treatments using the activated sludge process (Maszenan et al., 1997; Wei et al., 2020). This process relies on microbial activities to remove oxygen-consuming organics and nutrients like nitrogen and phosporus (Modin et al., 2016). Many strains within the *Amaricoccus* genus have the ability to reduce nitrate to nitrite and are capable of improving nitrogen removal at low C:N ratios (Kondo et al., 2009; Maszenan et al., 1997; Wang et al., 2020; Wei et al., 2020). This, however, would imply that *Amaricoccus* might be competing for nitrate with the plant by removing nutrients and reactive nitrogen from the biosphere, which is unlikely to occur within the root endosphere without causing visible symptoms to the plant. On the other hand, *Amaricoccus* spp. have been frequently described as poly-β-hydroxybutyrate (PHB)-producing organisms (Falvo et al., 2001; Kondo et al., 2009; Maszenan et al.,

1997). PHB is a polymer that can serve as an intracellular source of energy or carbon storage and is produced by microorganisms in response to physiological stress and limited nutrient conditions (Ackermann et al., 1995; Gillmaier et al., 2016; Obruca et al., 2018). Recent studies suggest that PHB accumulation is strongly interrelated with symbiosis initiation of nitrogen fixing bacteria to appropriately respond to nitrogen limitation in highly competitive environments (D'Alessio et al., 2017). PHB synthesizing bacteria within the root endosphere were shown to significantly correlate with an increase of the root area as well as the number of lateral roots (Alves et al., 2019). These functions could explain the significant higher abundance of *Amaricoccus* on low LUI during the plants vegetative phase (May). In this respect, the PHB cycle is a useful feature for these organisms to colonize *Dactylis glomerata* L. to overcome unfavorable conditions for plant growth. Conversely, despite their presence in various plant inner tissues and the rhizosphere, they have not been further analyzed with respect to plant growth-promoting traits.

The last group that was significantly affected by LUI in May was the family of *Acetobacteraceae,* which were, among not further classified taxa, assigned to the genera *Roseococcus* and *Roseomonas.* These are commonly found within plant inner tissues or rhizospheres and have previously been isolated from grapevines (Bruez et al., 2020), maize (Navarro-Noya et al., 2022; Renoud et al., 2022), poplar (Ulrich et al., 2008), rice (Chung et al., 2015), and several other plants. Nitrogen fixing bacterial representatives of *Acetobacteraceae* are widely known for their agricultural applicability, including *Roseococcus* and *Roseomonas* (Reis & Teixeira, 2015). Nitrogen is the essential limiting element for plant growth (Morgan & Connolly, 2013). It is a major component of chlorophyll, which is crucial for the conversion of light energy, water, and carbon dioxide into oxygen and chemical energy (i.e. photosynthesis), and of amino acids, which are the building blocks of proteins (Evans & Clarke, 2019; Morgan & Connolly, 2013). Furthermore, it is found in nucleic acids and other important biomolecules like adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), which are used in the light-independent reaction during photosynthesis (i.e. the Calvin Cycle; Evans, 1989; Walker et al., 2014). The biological process of nitrogen fixation transforms atmospheric nitrogen into inorganic compounds that can be utilized by the plant for the biosynthesis of nitrogen-containing organic compounds (Morgan & Connolly, 2013), thus enhancing plant performance under limited nitrogen availability. In addition to nitrogen fixation, *Roseococcus* and *Roseomonas* were reported to produce bacteriochlorophyll a (Sánchez-Porro et al., 2009; Yurkov et al., 1994). Bacteriochlorophylls are photosynthetic pigments that conduct photosynthesis, which harvest wavelengths of light that are not absorbed by plants or cyanobacteria, thereby increasing the spectral coverage for light absorption through sequestration by the eukaryotic water-soluble chlorophyll protein (Hitchcock et al., 2016). This might help the plant to

increase photosynthetic capacity, which in turn affects plant performance under nutrient limitations found in low LUI plots in May.

4.3.1.2 The reproductive stage

In June, which corresponds to the reproductive stage of the plant, not further classified *Hyphomicrobiales* (formerly *Rhizobiales*) were significantly increased under low LUI. It has been described that a large proportion of the order is highly adapted to a host-associated lifestyle, with many of them having important agricultural, ecological, and medical implications (Wang et al., 2020). Families within this order are largely associated with their biological nitrogen fixation abilities (Wang et al. 2020). However, through their high adaptability, *Hyphomicrobiales* are well-known for their diverse lifestyle, enabling a wide variety of plant beneficial traits like the supply of nutrients, phytohormones, or precursors of essential plant metabolites (Erlacher et al., 2015; Wang et al., 2020). The significant increase in abundance on low LUI compared to high LUI during the critical growth stage of when seeds are produced and high metabolic demands are exhibited, is reflected in the diversity of lifestyles and plant growth enhancing traits of members of *Hyphomicrobiales*. Furthermore, the results underline that the nitrogen-fixing abilities of members of *Hyphomicrobiales* also play an important role in perennial grasses.

Added to this, families of *Bryobacteraceae,* which were largely assigned to *Bryobacter* spp., are widely described as chemoheterotrophs that utilize various sugars and polysaccharides (Dedysh et al., 2017; Kulichevskaya et al., 2010). Members of *Cytophagaceae* are described as chemoorganotrophs that are frequently reported to utilize cellulose and the digestion of polysaccharides or proteins (McBride et al., 2014). As carbohydrates, or sugars, and the respective sugar signalling is essential to crucial processes required for plant growth, the metabolism of the plant needs tight coupling with regulatory mechanisms that control growth and development during flowering (Van den Ende, 2014). Sugar signals are reportedly generated either by carbohydrate concentration or by relative ratios to other metabolites, such as C:N ratio (Palenchar et al., 2004). Moreover, there is increasing evidence that sugars can crosstalk with phytohormone signalling networks to modulate critical growth phases and are able to regulate specific developmental processes (Eveland & Jackson, 2012). As a consequence, it is essential that the production, metabolism, and use of carbohydrates is carefully coordinated with the availability of photosynthates, i.e. the resulting products of photosynthesis, environmental factors, and the timing of important developmental growth processes. Therefore, the modulation and recycling of the carbohydrates, proteins, as well as the utilization of cellulose by endophytic members of *Bryobacteraceae* and *Cytophagaceae* can have profound effects

on plant growth, making these particularly important under low LUI during the plants reproductive stage.

As opposed to families significantly increased under low LUIs, *Turicibacteraceae,* which were further assigned to *Turicibacter* spp., were mostly found across high LUIs. *Turicibacter* spp. are commonly found in intestines of animals (Auchtung et al., 2016; Maki et al., 2020). They were possibly transferred through either fertilization with manure or grazing cattle or sheep on intensively managed sites, which is supported by the fact that they have not been reported as a plant growth-promoting taxon in other studies or as being extracted from plant inner tissues. On the other hand, *Turicibacteraceae* seem capable of entering the inner tissue of *Dactylis glomerata* without causing visible symptoms of disease. Considering this, the low abundance of this taxon suggests that they are sporadically acquired from the immediate environment (Santhanam et al., 2015).

4.3.1.3 The senescence stage

In October, where *Dactylis glomerata* plants were in the senescence stage, the phylogenetically closely related families *Sphingomonadaceae* and *Erythrobacteraceae* showed a significant increase in abundance on low LUI plots. Members of these taxa are characterized by the formation of glycosphingolipids in their outer cell walls, which are usually found in eukaryotes (Glaeser & Kämpfer, 2014; Tonon et al., 2014). In plants, sphingolipids are ubiquitous bioactive compounds of cells that have been implicated in many processes due to their wide range of physical properties, including membrane organization and dynamics, apoptosis, and senescence (Michaelson et al., 2016). Recent studies revealed that sphingolipids play a central role in signalling for programmed cell death that is an important process during plant senescence (Luttgeharm et al., 2015). Plant senescence is a degenerative process that occurs in a temporally coordinated manner and is the final phase of a developmental program that is linked to reproduction and survival (Woo et al., 2018). Unlike annual plants, which rely primarily on seeds as perennial structures, perennial plants survive adverse environmental conditions by maintaining viable storage organs such as roots and other plant organs (Sarath et al., 2014). Therefore, perennial plants have often developed mechanisms to recycle nutrients like nitrogen or photosynthates from shoots to maximize reproductive success by inducing seasonal dormancy and resume growth with the previously stored reserves as soon as conditions are favorable (Sarath et al., 2014; Yang & Udvardi, 2018). As nitrogen is the major limiting factor for plant productivity, it is crucial to optimize nitrogen use-efficiency through plant senescence. Therefore, the significant increase of the presence of sphingolipid producing families during the plant's senescence state on low LUI plots may alleviate and improve the efficiency of the process of programmed cell death and thus support the performance of the plant.

4.3.1.4 The rhizosphere and bulk soil

In contrast to the few families that were impacted by LUI within the roots, the number of affected taxa within the rhizosphere and bulk soil were higher, indicating a higher impact of LUI on the root surrounding area. In May, 16 families showed significant differences in the rhizosphere on low compared to high LUI. Among mostly low abundant taxa affected by LUI, *Comamonadaceae*, which is among the highest abundant families, were found in significantly higher amounts on high LUI plots. In addition, not further classified *Rhizobiales* were found to be increased under low LUI plots, which is in line with the root endosphere. In June, 23 low abundant families and in October, 32 families were affected by LUI. Among the latter were the two high abundant families *Xanthomonadaceae* and *Comamonadaceae,* both found in higher abundances on high LUI. Interestingly, *Oxalobacteraceae* were affected as well, which are further classified as *Massilia,* which is in line with the root endosphere. In the bulk soil, the effect of LUI was even more pronounced than in the rhizosphere, with 68 families being significantly different on high compared to low LUI plots, two of which being among the highest abundant families, *Sinobacteraceae* and *Hyphomicrobiaceae.* Interestingly*, Pseudomonadaceae*, which actually does not belong to high abundant taxa within the bulk soil but are the highest abundant family within the root endosphere, were found in higher amounts on low LUI plots. *Patulibacteraceae*, which were also found to be significantly impacted in the root endosphere in May, were impacted in the bulk soil as well. However, in contrast to the root endosphere, where *Patulibacteraceae* were found in higher abundance on low LUI plots, they were found in higher abundances on high LUI plots in bulk soil, underlining the plant's selective recruitment of beneficial taxa. In June, 59 families were affected, but not the ones that were found within the highest abundant families and in October, 34 families were influenced by LUI, with high abundant families *Xanthomonadaceae* and *Sphingomonadaceae* increased under low LUI plots.

Conclusively, these results indicate that the impact of LUI decreases with proximity to the plant root and while the impact of the plant increases from bulk soil to rhizosphere to the root endosphere. This in turn leads to a strongly distinctive bacterial community composition within the endosphere. The interaction of the plant and the surrounding soil is more pronounced in response to low nutrient availability, which in turn leads to a highly specific recruitment of beneficial bacteria throughout the different plant stages and proves more important on low LUI plots (Figure 5). Hence, these results are consistent with our expectations of the first part of the third hypothesis (**iii**) that bacterial composition in the endosphere is distinctive and specific to the nutritional and developmental needs of the plant, with LUI having an impact on nutrient availability, causing a feedback loop on low abundance taxa. This further is supported by the higher proportion and more even distribution of shared taxa between the root endosphere and

the surrounding rhizosphere and bulk soil, which is detected in the ternary plots (**Manuscript 1**).

4.3.2 Influencing factors on bacterial communities in the root endosphere

To further break down the extent of impact factors and confirm whether LUI is the strongest driver of changes compared to other soil properties, which was the aim of the second part of the third hypothesis (**iii**), a canonical analysis of principal coordinates was carried out. The LUI index is a continuous measure of land use intensity, which takes fertilization, mowing, and grazing into account, and has been shown to correlate with changes in soil nitrogen levels and soil parameters (**Manuscript 1 & 2**). Since differences in bacterial diversity and composition can be attributed to the different environmental parameters, the correlation of these was tested through the canonical analysis of the principal coordinates using soil characteristics as constraints to determine the driving forces of plant-associated bacterial assemblages. These were further used as explanatory constraints for the bacterial community composition within the root endosphere. In the present study, soil nitrate and ammonium were the strongest predictors for bacterial community structure (**Manuscript 1**). In response to nutrient deficiencies, plants change their root structure, attract microorganisms, and modify the chemical environment in the endosphere, as well as the rhizosphere (Hodges and Constable, 2010). The primary sources of nitrogen for plants have been suggested to be ammonium (NH_4^+) and nitrate (NO_3^- ; Tho et al. 2017). In dependence of the nitrogen source, plants respond differently as energy and reductants are required, thus, possibly causing a feedback loop on associated bacterial communities (Zhang et al. 2019). Ammonium, for example, is positively charged, therefore the plant releases a proton (H^+) for every NH_4^+ taken up, which leads to acidification of the rhizosphere (Stitt, 1999). Chemical changes, such as tissue undersupplied with essential cations like potassium, calcium, and magnesium, can in turn be triggered. (Britto and Kronzucker, 2002). Hence, if ammonium is the only source of nitrogen or is present in excessive amounts, this can lead to ammonium toxicity through ion disturbances in the plants, which in turn impairs plant growth and changes the composition of associated bacterial communities (Reference; Zhang et al. 2019). In contrast, nitrate is negatively charged, thus, the plant releases bicarbonate (HCO_3^-), which leads to an alkalization in the rhizosphere (Stitt, 1999). These changes in pH do not only change redox conditions within the rhizosphere but can influence the availability of other plant essential micronutrients (Zhang et al. 2019). Changes in pH were previously shown as the driving forces in changing soil microbial communities (Kaiser et al. 2016), which can be attributed to changes in the amount of available nitrogen and ammonium. Thus, is in line with the findings of the current study. Most plants, however, grow well when both nitrate and ammonium are available (Zhang et al., 2019), which also is reflected in the present study

on both high and low LUI plots. Since these results indicate that nitrate and ammonium levels are closely correlated with LUI index as they alter nutrient availability and affect soil properties (**Manuscript 1 & 2**), it was possible to verify the second part of hypothesis three (**iii**) that LUI has the greatest influence by altering soil properties, which in turn triggers a feedback loop on plant-associated communities (Figure 5). Other factors, such as gravimetric water content, C:N ratio, and water extractable carbon were shown to drive community composition to some extent, which is in line with previous studies (Brockett et al., 2012; Cederlund et al., 2014). The extent of the influence of the mentioned factors that were discussed in chapters 4.1 to 4.3 is schematically depicted in figure 5.

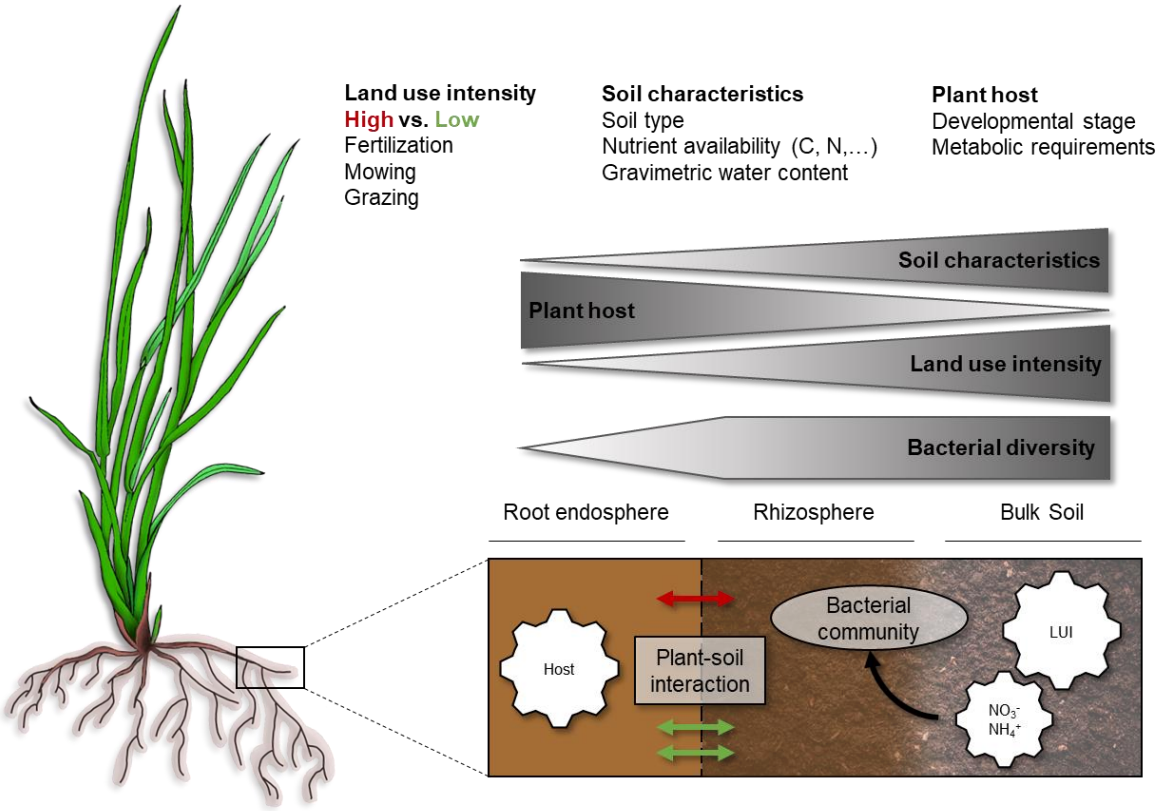

Figure 5: The impact on plant-associated bacterial communities and drivers of changes as identified within the current study.

Grey arrows indicate the influence of the mentioned factor or the bacterial diversity with respect to the compartment, whereas the cogs indicate the drivers of changes. The red/green arrows indicate a less strong plant-soil interaction on high (red) compared to low (green) LUI.

4.4 The bacterial core microbiome within the endosphere and rhizosphere

In this chapter, the impact of LUI on the formation of a specific plant-associated bacterial core composition at the root-soil interface will be discussed. Hypothesis four (**iv**) postulates the presence of a bacterial core microbiome with respect to the influence of LUI, which is the first part of hypothesis four, as well as a land use independent set of core bacterial keystone taxa, which is the second part of this hypothesis (**Manuscript 2**).

With respect to the first part of hypothesis four, it is expected that there will be a set of bacterial taxa, that will only be found in the core microbiome on high LUI or low LUI, respectively. For this purpose, a core microbiome was computed throughout all plant developmental stages on high versus low LUI. Furthermore, the stronger interaction of the plant with its surrounding soil at low LUI levels noted in previous chapters (see chapters 4.2 and 4.3) is expected to disentangle the close association of the plant with bacteria on high LUI in terms of their co-occurrence and the composition of core bacterial communities at the plant-soil interface (rhizosphere). Hence, it is expected that the structure of bacterial communities that contribute to the shared cores is less complex and variable in terms of lower bacterial diversity than at sites with extensive forms of land use (low LUI). Additionally, this effect will be more pronounced in the rhizosphere than in the root endosphere.

The second part of hypothesis four discusses the presence of a set of core taxa that will be present regardless of environmental factors and plant developmental stage, representing the most ecologically and functionally important bacterial associates with *Dactylis glomerata* L. Because these core taxa are persistent, ubiquitous, and are found in association with the host plant independently of LUI, they are classified as putative keystone species.

4.4.1 Bacterial variation of the bacterial core microbiome as influenced by land use intensity

In order to investigate a bacterial core assemblage with respect to the influence of LUI, which is the first part of hypothesis four, a core microbiome was computed throughout all plant developmental stages on high versus low LUI (**Manuscript 2**). Indeed, taxa were found that were only present within the core under high or low LUI, respectively. OTUs found exclusively in roots in all samples from low LUI sites included those attributed to *Caulobacter*. Several bacteria of this genus are frequently found as endophytes and have been reported to produce IAA, solubilize inorganic phosphate, and exhibit anti-fungal activity (Berrios, 2022; Chimwamurombe et al., 2016; Gao et al., 2021; Naveed et al., 2013). Some strains have also been described as nitrogen-fixing bacteria (Sithole et al., 2021), making them especially important under low LUI. This is supported by a study of Campisano et al. (2014), who found that

Caulobacter exhibited a higher abundance in grapevine cultivars under organic production compared to integrated pest management. On the other hand, a single OTU that was assigned to the genus *Labrys* was found in the core microbiome on high LUI. In addition to soil and sediment samples (Carvalho et al., 2008; Miller et al., 2005), species of this genus have also been isolated from rhizospheres of important crop plants (Fan et al., 2018) and Korean ginseng (Nguyen et al., 2015) and found as endophytes in *Clerodendrum colebrookianum* (Passari et al., 2016), *Phalaris arundinacea* (Węgrzyn & Felis, 2018), and legume nodules (Rangel et al., 2017; Tapia-García et al., 2020). Despite *Labrys* not being widely recognized as plant growth-promoting bacterium, it was identified to produce IAA and fix nitrogen (Tapia-García et al., 2020). Furthermore, it was shown to possess the gene *nodC*, which is associated with nodule formation in legumes (Tapia-García et al., 2020). In addition to this, *Labrys* strains that were isolated from sediment and soil have been shown to have the ability to reduce nitrate, assimilate various amino acids and sugars, have catalase activity etc. (Carvalho et al., 2008; Miller et al., 2005), which might also support plant growth under higher amounts of nitrate found on high LUI plots. Another OTU that was found in the core of high LUI only was *Agrobacterium*. Members of this genus are largely known for their ability for horizontal gene transfer of plasmid T-DNA into plant cells, leading to genetic modification of plants and thus making it a key player in genetic engineering (Nester, 2015). Several species within the *Agrobacterium* genus are known for their virulence as they carry the pathogenic capacities on the Ti (tumorigenic) or Ri (rhizogenic) plasmids. As a result, tumor-like growth can be induced and seed production is diminished, for example with crown-gall or hairy root disease (Lee et al., 2009; Zambryski et al., 1989). On the other hand, numerous avirulent *Agrobacterium* species have been found missing these plasmids (Bosmans et al., 2017; Nester, 2015). Moreover, *Agrobacterium* have been reported being among the most efficient plant growth-promoting bacteria through their ability to solubilize phosphate, fix nitrogen, produce siderophore, and enhance plant root development through phytohormone production (Pereira & Castro, 2014; Trivedi et al., 2020).

In the rhizosphere, several differences in the composition of core bacterial communities were found on high versus low LUI. These include OTUs assigned to *Variovorax*, which were only found in the core of low LUI samples. Species within this genus have also been reported to be among the most efficient plant growth bacteria through their metabolic diversity (Trivedi et al. 2020). Plant growth-promoting mechanisms include the production of various phytohormones, the reduction of plant stress, increasing nutrient availability, and providing protection from pathogenic infection (Han et al., 2011; Leadbetter & Greenberg, 2000; Pereira & Castro, 2014). The latter constitutes mechanisms related to their catabolic capacities and relies on the disruption of molecular communications of microbial communities. The best described mechanism to actively disrupt microbial signalling molecules, the *N*-acyl-*l*-homoserine lactones

(AHL), is the so-called “quorum quenching” and thus negatively impact species and inter-species communication that coordinates gene expression (Ghoul & Mitri, 2016). Quenching of bacterial cell-cell communication has been reported to act as putative biocontrol agents as they were shown to disrupt expression of virulence factors and biofilm formation of plant pathogens (Christiaen et al., 2011). Other species found exclusively in the core on low LUI were *Sphingomonas wittichii* and *Bosea genosp*. Numerous studies have shown that they can promote plant growth through the production of phytohormones (Khan et al., 2017; Shen & Fulthorpe, 2015). Furthermore, *Sphingomonas wittichii* was reported to metabolize a variety of aromatic compounds (Mydy et al., 2017), which has been shown to play an important role in bacterial colonization of the plant rhizosphere (Ledger et al., 2012). Moreover, other species belonging to the genera *Sphingomonas* and *Bosea* were shown to be diazotrophic, solubilize inorganic phosphorus, and can inhibit the growth of microbial pathogens, thus sustaining plant health and growth under scarcity of nutrients as found on low LUI plots (Correa-Galeote et al., 2018; De Meyer & Willems, 2012; Legein et al., 2020; Rilling et al., 2018). The last taxa found exclusively in the rhizosphere core on low LUI is *Asticcacaulis biprosthecium*, which is frequently found in the rhizosphere of different plants like maize (Walters et al., 2018), Populus (Bonito et al., 2019), or *Geum aleppicum* Jacq. (Zhu et al., 2014). However, it has not been further described in terms of plant growth-promoting traits. In contrast, on high LUI, OTUs belonging to *Rhizobium, Mycoplana, Labrys, Adhaeribacter, Paucibacter,* and *Microlunatus* were exclusively found in the core, all of which have been extracted from plant inner tissues and harboring plant growth-promoting traits (Egamberdieva et al., 2017; Egamberdiyeva & Höflich, 2003; Fernández-González et al., 2017; Lin et al., 2021; Nitin Parulekar et al., 2017; Oliveira Silva et al., 2020; Rilling et al., 2018; Trivedi et al., 2020).

As expected, OTUs found in the rhizosphere and soil samples differed, but all taxa detected in the soil core bacterial communities also occurred in the rhizosphere core under both LUIs. Differences in the soil core and rhizosphere may be attributed to the selective attraction of taxa by the plant. Interestingly, LUI influenced the proportion of OTUs contributing to the uniquely found OTUs (common in all plant development stages, but only at high or low LUI) and the number of taxa in the rhizosphere (total OTUs found in all plant development stages), as OTUs were assigned to more different taxa at high LUI. Although the predominant taxa specific to a plant stage were comparable in the rhizosphere, the amount and proportion of OTUs contributing to the core were lower under low LUI (156/19.4%) than under high LUI (235/25.35%). Added to this, the higher number of OTUs in the core bacterial communities associated with high LUI compared to those under low LUI suggests a higher variability and complexity of bacteria colonizing the rhizosphere throughout the different plant stages (Figure 6). Similar results were found for the root endosphere, albeit the numbers of OTUs that contribute to the core were comparable under high (12/13.64%) vs. low (14/11.4%) LUI, but a higher proportion

of core OTUs was found under high LUI compared to low (Figure 6). Hence, the effect of LUI appeared to be more pronounced in the rhizosphere compared to the root endosphere. This can be explained by the fact that under low nutrient availability, specific recruitment of beneficial microorganisms is crucial for the plant to improve its fitness and growth (Dreccer et al., 2013). Thus, a lower number of similar genera can be found, indicating a more selective control of *Dactylis glomerata* on soil bacterial communities at low LUI by plant exudation throughout the different plant developmental stages, which further underlines previous findings of the present study. Hence, we can verify the first part of hypothesis four (**iv**), that there are core taxa that are only found under high or low LUI, respectively, and that high LUI breaks the close link between the plant and its associated bacteria, which is seen to a higher extent in the rhizosphere compared to the root interior.

4.4.2 Putative keystone taxa that are independent of land use intensity

In order to identify putative plant-associated keystone taxa within the different plant compartments of *Dactylis glomerata* L., which is the second part of hypothesis four, the core microbiomes found under high versus low LUI were compared to find plant-associated bacterial taxa that are shared across all plant developmental stages under both LUIs, as these are likely to provide critical ecological functions. Indeed, plant-associated bacterial communities that were independent of plant developmental stage and LUI were found (**Manuscript 2**). OTUs that were assigned to *Pseudomonas veronii* were found in the core of the root endosphere, which underlines its importance for *Dactylis glomerata* L. As previously mentioned, species of *Pseudomonas* belong to the most efficient plant growth-promoting taxa, as they add a wide variety of beneficial traits to the plant holobiont (Trivedi et al., 2020). These include the production of various phytohormones such as IAA, cytokinins, or gibberillins, as well as nitrogen fixation and the production of antimicrobial compounds (Lodwig et al., 2003). Hence, they are numerously found in association with different plant hosts (Kloepper et al., 2013; Lally et al., 2017; Oteino et al., 2015; Rezzonico et al., 2005). *Pseudomonas veronii* in particular was shown to exhibit a high potential for biocontrol through increasing the availability of phosphate and ammonium in soil (Montes et al., 2016) and through the synthesis of IAA, thereby stimulating cell elongation and cell division of the plant, which is of major importance for the plant (Perrot-Rechenmann, 2010). In addition, high nematocidal activity was found for this species (Canchignia et al., 2016). This could be a critical attribute for *Dactylis glomerata* L., as it was reported that the abundance of plant-parasitic nematodes is significantly increased in *Dactylis glomerata* L. compared to other grasses and legumes (Viketoft et al., 2005). Furthermore, plant-feeding nematodes can cause a change in plant diversity and lead to spatial mosaics of plants in grasslands (Olff et al., 2000). Hence, high abundance of plant-feeding nematodes

can cause negative feedback on the plant, which in turn may significantly impact the competitiveness compared to other plant species. Other OTUs found to be putative keystone taxa in the root endosphere were assigned to *Rhizobium* and *Bradyrhizobium*, which are also known as being among the most important plant-beneficial bacteria (Trivedi et al., 2020). Both genera share a wide range of plant growth-promoting characteristics that include the production of phytohormones, siderophores, hydrogen cynide, exhibit antagonistic effects toward many plant pathogenic fungi (Chi et al., 2005; Egamberdieva et al., 2017; Taye et al., 2020), as well as the ability to fix nitrogen within nodules of leguminous plants (Fujita et al., 2014; Lodwig et al., 2003), and their ability to produce various phytohormones. Moreover, a high abundance of genes for nitrogen fixation of the endophytic *Rhizobium* spp. within inner tissues of the perennial grass sugarcane was found, suggesting their particular relevance for plant-associated nitrogen fixation in perennial grasses (Fischer et al., 2012). Due to its high abundance in the root endosphere, it presumably is a key player for plant health. Trivedi et al. (2020) even suggested that microbial taxa belonging to Rhizobiales and Pseudomonadales, which is the case for the putative keystone taxa in the current study, i.e. *Rhizobium* and *Pseudomonas veronii*, represent a universal core plant microbiota, which indicates a highly conserved adaptation to plant environments.

In the rhizosphere, more OTUs were found in total as well as in relative numbers as putative keystone taxa compared to the root endosphere. In total, 7 OTUs were classed as putative keystone taxa in the root, but 124 OTUs in the rhizosphere samples, indicating that these taxa are highly persistent and ubiquitous in agricultural soil. The higher number in the rhizosphere seems reasonable since there is no obstacle, such as penetrating the plant cell wall, to overcome. Interestingly, the most prominent genus found in the bacterial communities of the root core, i.e. *Pseudomonas*, was not as ubiquitous in the rhizosphere core under either LUI. This could be due to higher competition in the rhizosphere and the strong adaptation of *Pseudomonas* spp. to the specific conditions inside the root interior. Most OTUs found as putative keystone taxa within the rhizosphere were classified into the genera of *Rhodoplanes, Methylibium, Kaistobacter,* and *Bradyrhizobium*, which were also frequently isolated from other rhizosphere environments (Gkarmiri et al., 2017; Mao et al., 2014; Nakatsu et al., 2006; Rouws et al., 2014). Among those, *Bradyrhizobium* is the only genus that was also found as putative keystone taxa within the root endosphere under both LUI, which underlines its importance for the plant. Species within *Rhodoplanes* have been characterized as facultative photoorganotrophs and suggested to be involved in biological nitrogen fixation (Buckley et al., 2007; Hiraishi & Ueda, 1994). Representatives of *Methylibium* were described as facultative methylotrophs that actively utilize root exudates and degrade aromatic hydrocarbons and methyl tert-butyl ethers (Mao et al., 2014; Nakatsu et al., 2006). Members of *Kaistobacter* are also reported to be involved in the degradation of aromatic compounds and exhibit a high potential for pathogenic

disease suppression (Liu et al., 2016). These results indicate that *Dactylis glomerata* L. harbors a number of bacterial taxa that are highly likely to be keystone taxa, thus confirming the second part of hypothesis four (**iv**), that there are putative keystone taxa present in all samples, regardless of LUI levels, plant developmental stage, and other disturbing parameters. Hence, these taxa may be considered as specific to *Dactylis glomerata* L., which are important for the establishment of a healthy community composition and potentially crucial to the functioning of the plant-microbiome association. A schematic representation of chapter 4.4 can be found in Figure 6.

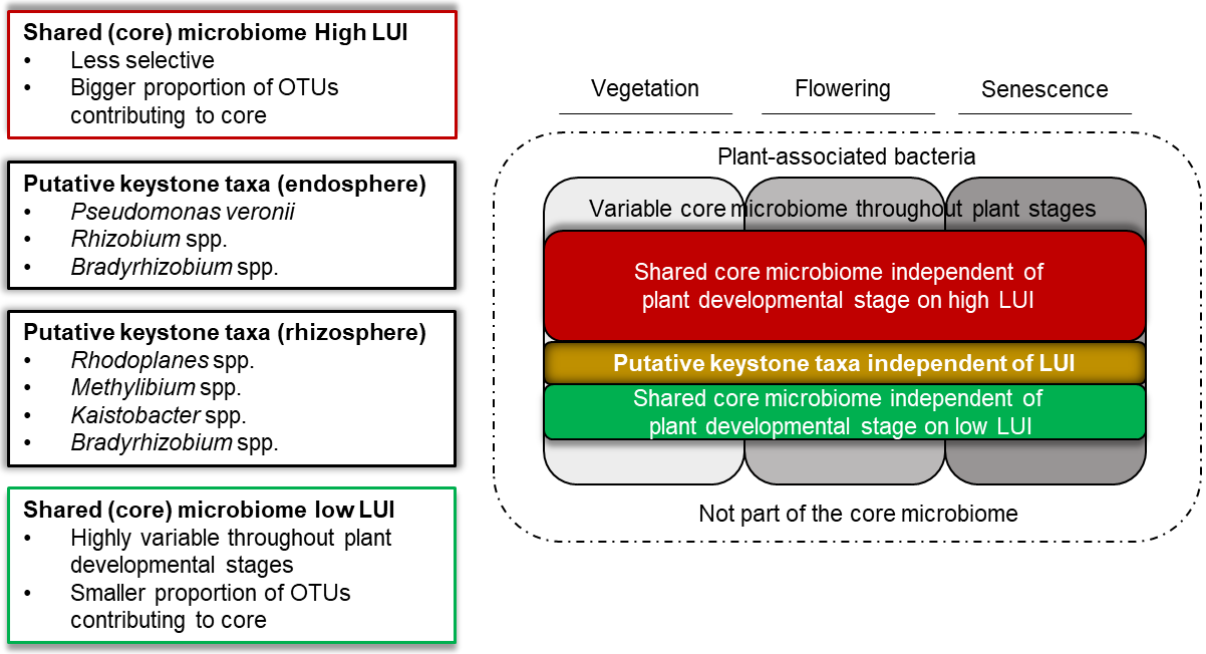

Figure 6: Schematic representation of components of the shared core microbiomes on high vs. low LUI as identified in this thesis.

Plant-associated bacteria that are not part of the shared microbiomes are comprised of occasionally occurring OTUs (dotted line). The grey boxes reflect the variable core microbiomes found specific to each plant developmental stage. The shared core microbiomes are depicted in red (high LUI) and green (low LUI) and reflect taxa that are found independent of plant developmental stage but dependent on LUI, i.e. found only on either high or low LUI. The size of the boxes indicates the proportion of the core microbiome among the total amount of shared OTUs. The putative keystone taxa, which are found independent of plant developmental stage and LUI are depicted in yellow, i.e. found in the core microbiomes on both LUIs.

Conclusion and Outlook

Although plant-associated bacteria play a crucial role in enhancing and promoting plant growth and provide resilience against environmental and anthropogenic stress factors, our knowledge about the impact of LUI on plant-associated bacteria is still limited. The current study provides first insights into the complex response of plant-associated bacterial communities of the perennial grass *Dactylis glomerata* L. towards a continuous LUI measure during different plant developmental stages using 16S rRNA high-throughput metabarcoding.

The results suggest that the influence of LUI on bacterial diversity and community composition is most pronounced in bulk soil and the rhizosphere. In contrast, the effect of LUI in the root endosphere is less pronounced, which is in line with our first hypothesis (**i**). In bulk soil, β-diversity measures for both abundance and the presence/absence of certain taxa were affected significantly by LUI, whereas in the rhizosphere, only presence/absence was impacted in all plant developmental stages. In contrast, in the root endosphere, a significant influence of LUI was detected only in the presence/absence of taxa with low abundance in the reproductive stage of the plant. In addition, the magnitude and direction of shifts in abundance and presence/absence of individual OTUs in response to LUI increased from the roots, where they were the lowest, to the rhizosphere to the bulk soil and are dependent on plant developmental stage, as reflected by the significance level and variance for both β-diversity measures.

These results suggest that the plant is the most important selective force in shaping the diversity and composition of associated communities. In turn, these findings imply that the influence of LUI decreases with proximity to the plant root, leading to a relatively stable, consistent, and very distinct composition of bacteria in the root endosphere that is mainly influenced by the plant. Analysis of β-diversity and the community structure confirmed these results, as the assemblage patterns in the root endosphere strongly differed to the rhizosphere and bulk soil, which verified the objective of the second hypothesis (**ii**).

Despite the stable community composition within the plant endosphere, the current study identified a feedback loop on endophytic communities during the plant's reproductive stage. Shifts in community composition could mainly be attributed to nitrate and ammonium, which were closely correlated with the LUI index in the current study, as they altered nutrient availability and shape soil properties. In this regard, LUI was identified as a major driver of changes in community composition in the root endosphere of *Dactylis glomerata* L., which verified the objective of the third hypothesis (**iii**). As a result, the interaction between plant and surrounding soil is more pronounced at low LUI than at high LUI, as nutrient availability forces the specific recruitment of beneficial bacteria in response to metabolic demands during the different stages of plant development.

Although the associated communities were dynamically shaped by the plant, a set of putative keystone taxa was identified, which were present regardless of plant developmental stage and LUI. Genera consistently found in the root endosphere belonged to *Pseudomonas, Rhizobium, and Bradyrhizobium*. In the rhizosphere, the majority of genera identified as keystone bacterial communities were assigned to *Rhodoplanes, Methylibium, Kaistobacter,* and *Bradyrhizobium*. Their persistent presence independent of LUI or growth stage, as well as their plant growth-promoting properties, may provide initial insight into the composition of a vital part of the plant microbiome. Additionally, when comparing the shared core microbiomes under high vs. low LUI, a greater compositional variation across different sampling dates was observed at low LUI versus high LUI, underlining the greater adaptation of plant-associated bacteria under low LUI. These results are in line with our expectations of hypothesis four (**iv**), that the bacterial structure is more complex and dynamic on low LUI levels, but that there are a set of bacterial taxa found across all plant developmental stages and LUI levels. Moreover, these results were significant considering the minimum cut-off of 0.001%, i.e. the required number of reads for an OTU was 103 after the cut-off. Given the functions associated with the discovered bacterial species, we could speculate that the main functions of the plant-associated shared core bacterial community might be related to plant growth and stress response in particular.

Albeit the keystone taxa identified in the present study have been associated with plant growth-promoting properties in previous studies, no conclusion can be drawn from the measured co-existence of plant-associated bacterial communities in different LUIs as to how the discovered species react and interact with their host plants and other microbes. The effects of land use intensification on plant-associated bacterial communities requires additional consideration of the interrelationships between processes performed by key players. Effects include microbially mediated processes, metabolic pathways, and proteins associated with colonization (Trivedi et al., 2020). Several mechanisms are observed, ranging from cooperative to competitive interactions (Schlechter et al., 2019). To take a first step towards identifying correlations, analyses of the diverse and complex interactions between microorganisms necessary for the selective establishment of plant-associated microbiomes in response to land use intensification are required (Trivedi et al. 2020). The identification of co-occuring (core) bacterial networks of plant-associated microorganisms should be assessed to account for the presence and contribution of microorganisms to the holobiont, which may provide useful insights into the influence of biotic interactions with the host or with other bacterial taxa and their contribution to ecosystem functions. However, co-occurrence analyses may not be sufficient to interpret species interactions as they do not indicate causal relationships through relying on species abundance but may provide an opportunity to investigate possible causal relationships of hub species with other species to understand how the behaviour of keystone taxa can be validated experimentally (Trivedi et al. 2020). These hub species could possibly have a regulatory effect on the co-

occurrence network. The removal of highly connected taxa may alter the microbial composition significantly, which may result in the loss of interactions and is likely to collapse microbial populations and functions (Niu et al., 2017). Therefore, the analysis of co-occurrence networks of plant-associated communities would provide an important baseline for possible microbe-microbe and microbe-plant interactions to further allow deeper investigation on identified positive or negative correlations of co-occurring taxa and their respective functional traits to reveal their functional potential. This could lead to a deeper understanding of how different land use gradients affect the functional potential of plant-associated bacteria and will provide important insights for sustainable grassland management and help ensure associated ecosystem functions and services. In addition, although our study revealed the existence of common plant-associated bacterial taxa under different land use intensities, these results were specific to *Dactylis glomerata* L. in the grassland soils studied. Therefore, it would be beneficial to also evaluate bacterial and other microbial communities associated with different species of *Dactylis glomerata* or to study wider biogeographic patterns of the root-associated microbiome of *Dactylis glomerata* L. or other grass species of the *Poaceae*.

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List of abbreviations

16S rRNA	Component of the prokaryotic ribosomal small subunit
18S rRNA	Component of the eukaryotic ribosomal small subunit
ATP	Adenosine triphosphate
°C	Degree Celsius
µg	Microgram
µM	Micromolar
ABA	Abscisic acid
ADONIS	Permutational multivariate analysis of variance test
AHL	*N*-acyl-*l*-homoserine lactones
ANOVA	Analysis of variance
BEO	Biodiversity Exploratories
bp	Base pair
BSA	Bovine serum albumin
C	Carbon
C:N	Carbon:nitrate ratio
Ca	Calcium
CCA	Canonical correspondence analysis
cm	Centimeter
CO_2	Carbon dioxide
DEPC	Diethyl dicarbonate
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dw	Dry weight
e.g.	For example
EPs	Experimental Plots
FAO	Food and Agriculture Organization of the United Nations
Fe	Iron
g	Gram
H^+	Proton/cationic form of atomic hydrogen
ha	Hectar
HCO_3^-	Bicarbonate
i.e.	This means
IAA	Indole-3-acetic acid
K	Potassium
kg	Kilogram

km^2	Square kilometer
LUI	Land use intensity
m	Meter
M	Molar
Mg	Magnesium
mg	Milligram
ml	Milliliter
mm	Millimeter
mRNA	Messenger RNA
N	Nitrogen
NADP+	Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$-Pi	Inorganic bicarbonate
$NaHCO_3$-Po	Organic bicarbonate
NaOH-Pi	Inorganic sodium hydroxide
NaOH-Po	Organic sodium hydroxide
NB-agar	Nutrient agar
NCBI	National Center for Biotechnology Information
ng	Nanogram
NH_4^+	Ammonium
NH_4-N	Ammonium-bound nitrogen
nif	Genes encoding enzymes involved in the fixation of atmospheric nitrogen
nM	Nanomolar
nmol	Nanomol
NO_2^-	Nitrite
NO_3^-	Nitrate
NO_3-N	Nitrate-bound nitrogen
nod	Genes encoding nodulation factors
OTU	Operational taxonomic unit
P	Phosphorus
PAH	Polycyclic aromatic hydrocarbons
PBS	Phosphate-buffered saline
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
PD	Phylogenetic diversity
PEG	Polyethylene glycol
PGPB	Plant growth promoting bacteria
pH	Potential of hydrogen/measure of acidity or basicity

PHB	Poly-β-hydroxybutyrate
pmol	Picomol
QIIME	Quantitative Insights Into Microbial Ecology
Ri	Rhizogenic
RNase	Ribonuclease
rRNA	Ribosomal ribonucleic acid
SINA	SILVA Incremental Aligner
spp.	Species pluralis
SRA	Sequence Read Archive
SSU	Small subunit
T-DNA	Transfer Deoxyribonucleic acid
Ti	Tumorigenic
TNS	Trypsin Neutralization Solution
UNEP	United Nations Environment Programme
UNESCO	United Nations Educational, Scientific and Cultural Organization
V1-V9	Hypervariable regions
VIPs	Very intensive plots
vol/vol	Volume fraction
vs.	Versus
w/v	weight per volume
WEOC	Water extractable organic carbon
WEON	Water extractable organic nitrogen

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Appendix

Manuscript 1

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ORIGINAL RESEARCH
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The Influence of Land Use Intensity on the Plant-Associated Microbiome of *Dactylis glomerata* L.

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In this study, we investigated the impact of different land use intensities (LUI) on the root-associated microbiome of *Dactylis glomerata* (orchardgrass). For this purpose, eight sampling sites with different land use intensity levels but comparable soil properties were selected in the southwest of Germany. Experimental plots covered land use levels from natural grassland up to intensively managed meadows. We used 16S rRNA gene based barcoding to assess the plant-associated community structure in the endosphere, rhizosphere and bulk soil of *D. glomerata*. Samples were taken at the reproductive stage of the plant in early summer. Our data indicated that roots harbor a distinct bacterial community, which clearly differed from the microbiome of the rhizosphere and bulk soil. Our results revealed *Pseudomonadaceae*, *Enterobacteriaceae* and *Comamonadaceae* as the most abundant endophytes independently of land use intensity. Rhizosphere and bulk soil were dominated also by *Proteobacteria*, but the most abundant families differed from those obtained from root samples. In the soil, the effect of land use intensity was more pronounced compared to root endophytes leading to a clearly distinct pattern of bacterial communities under different LUI from rhizosphere and bulk soil vs. endophytes. Overall, a change of community structure on the plant–soil interface was observed, as the number of shared OTUs between all three compartments investigated increased with decreasing land use intensity. Thus, our findings suggest a stronger interaction of the plant with its surrounding soil under low land use intensity. Furthermore, the amount and quality of available nitrogen was identified as a major driver for shifts in the microbiome structure in all compartments.

Keywords: ***Dactylis glomerata*, land use change, endophytes, rhizosphere, soil microbiome, biodiversity**

INTRODUCTION

Numerous studies have shown that healthy plants can host up to a few thousand different microbial species in their different organs, including roots, stems and leaves (Berendsen et al., 2012; Bulgarelli et al., 2012; Hacquard et al., 2015). Plant-associated microbes benefit from their host, as plants provide easily degradable carbon and a protected environment as well as physical structures for colonization (Kowalchuk et al., 2002). Vice versa, the plant-associated microbiome positively

influences plant growth by supplying nutrients like nitrogen or phosphorus, improving stress tolerance and protecting plants from phytopathogens (Martinez-Viveros et al., 2010; Berendsen et al., 2012; Gaiero et al., 2013; Reinhold-Hurek et al., 2015).

The various plant compartments colonized by microbes do not provide constant environments, but change with plant developmental stage or as a result of environmental factors including temperature, water content, or nutrient availability (Kowalchuk et al., 2002; Tuteja and Sopory, 2008; Philippot et al., 2013). The importance of environmental factors as drivers for the plant phenotype has been confirmed in many studies, where the same plant genotype developed different phenotypes in response to differing environmental conditions (e.g., Schlichting, 1986; Sultan, 2000; Valladares et al., 2007), which in turn also favored the development of distinct microbial communities colonizing plant compartments (Compant et al., 2005; Gnanamanickam, 2006; Ernebjerg and Kishony, 2012; Gaiero et al., 2013; Tardieu, 2013; He et al., 2014). However, most of these studies were performed under greenhouse conditions, where key factors influencing plant performance were kept in the optimum and only one factor was changed. Wagner et al. (2016) postulated that the plant genotype plays only a minor role in the establishment of root-associated microbiomes under natural conditions in contrast to greenhouse experiments. Thus studies under natural conditions are needed as a next step to evaluate the importance of various multifactorial scenarios for the plant phenotype and plant-associated microbiomes.

Land use intensity strongly influences a large number of environmental factors including the amount and quality of nutrients, the soil structure and general biodiversity pattern (Birkhofer et al., 2012). Thus, land use intensification is a typical example for a driver inducing multifactorial changes in a given plant phenotype (Kirkham et al., 1996; Wedin and Tilman, 1996) and may consequently strongly modulate plant-associated microbial communities. Therefore, in this study we investigated the impact of different levels of land use intensity in grassland ecosystems on the root-associated microbiome of orchardgrass (*Dactylis glomerata* L.), a perennial forage grass which occurs under a wide range of land use intensity levels at grassland sides of Central Europe. Root-associated microbiomes were separated into (a) root endophytes and (b) microbes colonizing the rhizosphere. Furthermore, we investigated the impact of different levels of land use intensity on the microbiome of the bulk soil (c). We postulated that any effects of land use intensity will be most pronounced in the bulk soil and in the rhizosphere, whereas effects on root endophytes would be less pronounced and mainly modulated by the performance of the plants. We further hypothesized that in all investigated compartments land use intensity is negatively correlated with microbial diversity.

MATERIALS AND METHODS

Sites Description and Sample Collection

This study was performed within the framework of the German Biodiversity Exploratories, a project investigating large-scale and long-term relationships of biodiversity and land use in Central European grasslands (Fischer et al., 2010). The Biodiversity Exploratory "Schwäbische Alb," is located in the low mountain ranges in the southwest of Germany and covers an area of about 422 km^2 including 100 grassland and forest experimental plots with different land use intensities (LUI). Plots are classified along a land use intensity index according to Blüthgen et al. (2012). As the index is based on three different management components (fertilization intensity, mowing frequency and grazing level) which are equally weighted, it provides a unique design for detailed analysis of long term effects on biota, as inter-annual variations in management practices are normalized. Eight plots were selected for this study. Mowing regime, fertilization and grazing management of these plots ranged from unfertilized meadows and pastures to highly fertilized meadows and mown pastures, resulting in a classification of four plots with high and four plots with low land use intensity, which were treated as true replicates (Plot IDs intensive LUI: AEG6, AEG19, AEG20, AEG21; Plot IDs extensive LUI: AEG7, AEG28, AEG33, AEG34; see Supplementary Table S1). The soil type of all plots has been characterized as Rendzic Leptosol (according to the FAO classification system). Mean annual temperature in the region is 9.9°C and mean annual precipitation is in the range of 730 mm.

Nine plants of *D. glomerata* per plot were collected in an area of 1.5 m × 1.5 m in June 2015 and pooled into three replicates to obtain enough material for further analyses (3 plants per replicate, that were closest together). All selected plants were in the reproductive state (production of seeds) without any disease symptoms like leaf spots. Plants were excavated carefully with adhering rhizosphere and bulk soil. Soil which could be shaken off easily from the roots was defined as bulk soil. The remaining soil that firmly adhered to the root was defined as rhizosphere. Bulk soil and root samples with adhering rhizosphere were collected in 15 ml tubes for DNA extraction, immediately frozen on dry ice and stored at −80°C until further analyzed. Above-ground biomass of the plant was harvested by cutting leaves with sterilized scissors and frozen immediately (−20°C). Additional bulk soil samples were stored at 4°C and sieved for soil carbon/nitrogen and dry weight measurement.

Soil and Plant Carbon and Nitrogen Content

Dissolved carbon and nitrogen content in soil was measured after extraction of 5.0 g fresh soil using 0.01 M calcium chloride after shaking on a horizontal shaker for 45 min followed by centrifugation (2 min at 4500 x *g*). The extracts were filtered through a Millex HV Millipore filter, pore size, 0.45 µm (Merck, Darmstadt, Germany). Finally, water extractable organic carbon (WEOC) and nitrogen (WEON) were measured on DIMA-TOC 100 (Dima Tec, Langenhagen, Germany). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) were determined by continuous flow analysis using a photometric autoanalyzer (CFA-SAN Plus; Skalar Analytik, Germany).

Above-ground plant material was dried at 65°C for 2 days prior to pulverization in a Tissue LyserII (Qiagen GmbH, Germany). About 1.5 mg of the pulverized material was weighted into 3.5 mm × 5 mm tin capsules (HEKAtech GmbH, Wegberg,

Germany). Total carbon and nitrogen contents were determined using an Elemental-Analysator 'Euro-EA' (Eurovector, Milano, Italy).

Separation of Microbes from the Rhizosphere and Root Interior

Dactylis glomerata roots were transferred to 15 ml falcon tubes containing 7.5 ml sterile 1× PBS amended with 0.02% Silwet (PBS, AppliChem, Darmstadt; Silwet L-77) and shaken at 180 rpm for 5 min to remove the rhizosphere soil. This step was repeated three times by transferring the roots to new 15 ml falcon tubes containing sterile 1× PBS-S. The PBS-S buffer was subsequently centrifuged and the resulting pellets were frozen in liquid nitrogen and stored at −80°C.

After rhizosphere removal, roots were submerged in sterile 1% Tween 20 for 2 min, washed in sterile water, incubated for 2 min in 70% ethanol and subsequently rinsed three times with sterile distilled water. Afterward, roots were surface sterilized using 5% sodium hypochlorite for 10 min, washed eight times with sterile distilled water, frozen in liquid nitrogen and stored at −80°C. The disinfection efficiency was confirmed by the absence of PCR products after amplification of the 16S rRNA genes using DNA extracted from the final wash water as a template (data not shown). Additionally, no colonies were obtained when 200 µl of the final wash water were plated onto NB-agar plates and incubated for 10 days on 28°C (data not shown).

Nucleic Acid Extraction

DNA was extracted using a phenol-chloroform-based method according to Lueders et al. (2004). We used surface sterilized roots, rhizosphere- and bulk soil samples taken from eight plots (3 replicates per plot) resulting in 72 samples in total. Briefly, root samples were frozen in liquid nitrogen and pre-homogenized in a TissueLyser II (Qiagen GmbH, Germany) prior to DNA extraction. 0.1 g of roots and 0.3 g of rhizosphere- and bulk soil, respectively, were homogenized in lysing matrix tubes E (MP Biomedicals, France) in 120 mM sodium phosphate buffer (pH 8) and TNS solution [500 mM Tris–HCl pH 8.0, 100 mM NaCl, 10 % SDS (wt/vol)] and centrifuged at 16,100 × *g* for 10 min at 4°C. The supernatant was removed and successively mixed with an equal amount of Phenol/Chloroform/Isoamyl Alcohol (25:24:1, Sigma–Aldrich) and Chloroform/Isoamyl Alcohol [(24:1 (vol/vol)] and centrifuged for 5 min at 16,100 × *g*. The DNA was precipitated using 30% (wt/vol) polyethylene glycol (PEG) solution [PEG 6000, NaCl], followed by 2 h of incubation on ice. After centrifugation (16,100 × *g*, 10 min, 4°C), the pellet was washed in ice-cold DNase/RNase free 70% ethanol, air-dried, and eluted in 30 µl 0.1% diethylpyrocarbonate water. The DNA concentration was quantified in duplicates using the Quant-iTPico™ Green® ds DNA assay Kit (Invitrogen, Carlsbad, CA, United States) according to the manufacturer's instructions. Measurements were performed with a SpectraMax Gemini EM Fluorescence Plate Reader Spectrometer (Molecular Devices, Sunnyvale, CA, United States). Values were corrected for background fluorescence by addition of negative controls. DNA extracts were stored at −80°C until further use.

PCR Amplification and Illumina Sequencing

Next generation sequencing was performed using the Illumina MiSeq platform (Illumina Inc., San Diego, CA, United States). Library preparation was accomplished according to the "16S Metagenomic Sequencing Library Preparation" protocol proposed by Illumina Inc., San Diego, CA, United States. Briefly, polymerase chain reaction (PCR) of the 16S rRNA region was performed in triplicates using the primers 335Fc (5′-CADACTCCTACGGGAGGC-3′) and 769Rc (5′-ATCCTGTTTGMTMCCCVCRC-3′) published by Dorn-In et al. (2015) with Illumina adapter sequences. The reaction mix contained of 12.5 µl NEB Next High Fidelity Master Mix (Illumina Inc., United States), 0.5 µl of each primer (10 pmol/µl), 2.5 µl of 3% BSA, 100–200 ng of template DNA and ad DEPC water 25 µl. PCR conditions were the following: 98°C for 5 min, followed by 20 cycles for rhizosphere and bulk soil samples and 28 cycles for roots samples, respectively, at 98°C for 10 s, 60°C for 30 s and 72°C for 30 s, followed by 72°C for 5 min. Each PCR was performed in triplicates to minimize PCR errors. Negative control samples using DEPC water instead of template DNA were treated similarly. DNA amplicons were analyzed using a 2% agarose gel, triplicates were pooled and subsequently purified using the Agencourt®AMPure®XP (Beckman Coulter Company, United States) extraction kit according to manufacturer's instructions. However, the ratio of AMPure XP to PCR reaction was adapted to 0.6 to 1. Amplicon sizes and presence of primer-dimers were checked using a Bioanalyzer 2100 (Agilent Technologies, United States), the DNA 7500 kit (Agilent Technologies, United States) and quantified using the Quant-iT PicoGreen kit (Life Technologies, United States). Afterward, indexing PCR was performed using 12.5 µl NEB Next High Fidelity Master Mix, 10 ng DNA of the previous PCR products and 10 pmol of each primer containing adapter overhangs. For each indexing PCR the annealing temperature was reduced to 55°C and the number of cycles set to eight cycles. Purified PCR products were pooled in equimolar ratios to a final concentration of 4 nM and sequenced using the MiSeq Reagent kit v3 (600 cycles) (Illumina Inc., United States) for paired-end sequencing. Sequence files were deposited in the NCBI Sequence Read Archive under accession number SRP102620.

Sequence Data Analysis

Sequences were analyzed using QIIME (Quantitative Insights into Microbial Ecology) software package version 1.9.1 (Caporaso et al., 2010) and default parameters. FASTQ files were trimmed and merged with a minimum read length of 50 and minimum Phred score of 15 using AdapterRemoval (Schubert et al., 2016). After removal of chimeric sequences, quality and length filtering (400–480 bp) of the merged reads with DeconSeq (Schmieder and Edwards, 2011), sequences were clustered into operational taxonomic units (OTUs) at 97% sequence identity with an open reference strategy using the GreenGenes database (13_5 release) as a reference (DeSantis et al., 2006). Subsequently, taxonomic classification was carried out using RDP classifier (Wang et al., 2007) retrained on the GreenGenes 16S rRNA reference database.

The output was filtered with an abundance cut-off of 0.005% to remove singletons. A core set of QIIME diversity analyses was run on rarefied data for categories to compare (compartment and land use intensity). Faith's phylogenetic diversity was used as a measure for α-diversity. Statistical significance in the global bacterial community composition was determined by permutational multivariate analysis of variance (ADONIS). Differences between LUIs among families were tested using *t*-test. The core microbiome was computed via QIIME and visualized with the Bioinformatics & Evolutionary Genomics webtool[1]. Ternary plots were constructed with the R package "vcd" using rarefied OTU tables. Constrained principal coordinate analyses were performed in R using the R package "vegan." Further statistical analyses and visualization of the data were carried out in R using the packages "ggplot2," "plyr," "ade4," and "ape."

RESULTS

Soil Characteristics

Water extractable organic carbon values were evenly distributed among different LUI levels. In contrast, WEON, nitrate, and ammonium concentrations changed with LUI levels. WEON and nitrate decreased from intensively to extensively managed sites. While WEON and nitrate levels were significantly higher in soil samples from the high LUI sites (Supplementary Table S1), ammonium concentrations were significantly higher under low land use (from 2.9–0.1 $\mu g\, N\, g^{-1}$ dw). Plant carbon/nitrogen ratio increased from high to low land use intensity levels. The highest value (24.31 C/N) was detected on extensively managed sites.

Characterization of Bacterial Diversity of Bulk Soil, Rhizosphere and Root Interior

A total of 3,883,552 raw reads were obtained. After quality filtering and chimera check with DeconSeq 3,853,784 remained, which could be clustered into a total of 3,241 OTUs at 97% sequence similarity. Afterward, plant derived 16S sequences (displayed by *Cyanobacteria* that were assigned to chloroplasts) were removed and output was filtered with an abundance cut-off of 0.005%, resulting in 2,903,916 reads. To make results comparable, the data set was rarefied to 11,052 reads per sample, reflecting the lowest obtained read number per sample. Rarefaction curves indicated that sampling depths were sufficient as plateaus were reached for samples from all compartments (Supplementary Figure S1).

As expected, α-diversity (**Figure 1**) was significantly lower in root samples ($p < 0.05$) compared to rhizosphere and bulk soil, respectively, for both LUIs. β-diversity indicated a clear separation of bacterial communities originating from root compared to rhizosphere and bulk soil, explaining up to 33% (weighted and unweighted Unifrac metrics) of the overall variance in the data (Supplementary Figure S2).

Phylogenetic classification of the obtained OTUs revealed 18 phyla, highlighting *Proteobacteria* as the dominating phylum in all compartments with increasing numbers from bulk soil (60%) and rhizosphere (69%) to roots (91%) (Supplementary Table S2A). In roots OTUs linked to *Pseudomonadaceae* were most abundant (15%). Other abundant OTUs in roots were linked to *Enterobacteriaceae* (12%), *Comamonadaceae* (10%), *Oxalobacteraceae* (9%), *Rhizobiaceae* (9%), *Sphingomonadaceae* (7%) and *Xanthomonadaceae* (6%). In contrast, in the rhizosphere, *Sinobacteriaceae* (9%), *Hyphomicrobiaceae* (8%), *Comamonadaceae* (7%), *Chitinophagaceae* (7%) and *Xanthomonadaceae* (6%) were found to be the most abundant families, while bulk soil was dominated by *Chitinophagaceae* (12%), *Hyphomicrobiaceae* (10%) and *Sinobacteriaceae* (9%) (Supplementary Table S2B). Besides *Proteobacteria*, *Bacteroidetes* (20/13/5%), *Actinobacteria* (9/10/2%) and *Acidobacteria* (4/4/1%) were also abundant in all compartments.

The Influence of Land Use Intensity on Plant-Associated Bacterial Diversity

Analysis of α-diversity revealed a significant impact of LUI on bacterial diversity in bulk soil as well as in roots ($p < 0.05$), with higher α-diversity values in bulk soil and lower α-diversity in the root interior under high LUI (**Figure 1**). Effects on the microbiome diversity in the rhizosphere in response to LUI were not significant ($p > 0.05$). Furthermore, significant changes in β-diversity in response to land use intensity were revealed by permutational multivariate analysis of variance (ADONIS) using qualitative measures (unweighted Unifrac distance matrix) for all three compartments. In contrast, no significant difference was detected on Bray–Curtis dissimilarity or weighted Unifrac distance in roots and rhizosphere, leading to the assumption that significance is a result of presence and/or absence of certain OTUs. However, in bulk soil LUI also significantly affected community composition in relative abundance (Bray–Curtis; Supplementary Table S3).

To identify changes in bacterial communities as a consequence of different LUIs in the three compartments, ternary plots were prepared (**Figure 2**). The results reveal that roots harbored distinct communities under both LUI. However, a shift in community structure can be observed as a consequence of land use. At high LUI, a large number of OTUs was almost exclusively shared between rhizosphere and bulk soil and was not found in the root interior. At low LUI a remarkable amount of OTUs could be found in the root interior and in the rhizosphere suggesting a specific recruitment of bacterial taxa by the plant.

To gain deeper insights into the shared communities in all compartments (OTUs present across 95% of the samples) under low or high LUI, respectively, an analysis of the core microbiome was performed. Consistent with the ternary plot, more shared OTUs were found under low LUI (38 OTUs) compared to high LUI (17 OTUs). Among these, a shared core of 12 OTUs could be defined which was independent of compartment and LUI (Supplementary Figure S3). These OTUs were assigned to *Bradyrhizobiaceae* (4 OTUs), *Sphingomonadaceae* (2 OTUs), *Pseudomonadaceae* (1 OTUs), *Comamonadaceae* (1 OTU), *Hyphomicrobiaceae* (2 OTUs), *Caulobacteraceae* (1 OTUs), and *Rhizobiaceae* (1 OTU). OTUs shared under low or high LUI are presented in Supplementary Tables S4–S6.

[1]http://bioinformatics.psb.ugent.be/webtools/Venn/

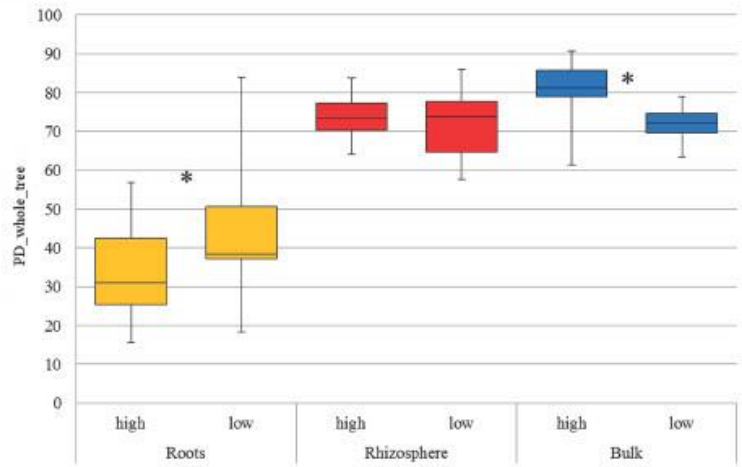

FIGURE 1 | Faith's phylogenetic diversity of all compartments under low and high land use intensity, respectively. The distribution of phylogenetic diversity is shown at 97% sequence similarity. The boxplot indicates the first and third quartile. The median is indicated as horizontal line and whiskers indicate minimum or maximum, respectively. Significant differences among land use intensities are indicated with an asterisk (*t*-test, $p < 0.05$).

Significant differences with respect to LUI in the abundance of assigned families in roots could only be detected for four taxa (Supplementary Table S7A). Two families assigned to *Bryobacteraceae* and *Cytophagaceae* and unassigned members of *Rhizobiales* were more present under low LUI while one taxon was more prominent under high LUI (*Turicibacteraceae*). In contrast, 19 families were found to significantly differ among LUIs in the rhizosphere (Supplementary Table S7B) and 48 in bulk soil, respectively (Supplementary Table S7C; *p*-value < 0.05). Interestingly, only two of the taxa that were significantly affected by LUI in roots appeared to be also affected in the other compartments. *Turicibacteraceae* were influenced in bulk soil as well as unassigned members of *Rhizobiales* in rhizosphere ($p < 0.05$). However, LUI did not have a significant impact on the most abundant families colonizing the root interior.

To better understand the driving factors of the differences in bacterial community structure in the three compartments, we performed a canonical analysis of principal coordinates. Including the soil characteristics in the ordination revealed that LUI is best represented by nitrate (high LUI) and ammonium (low LUI). The soil characteristics used explained 36% of the variation of microbial community structure in the roots (**Figure 3A**). Apart from compartment, LUI and WEON appeared to be the main driving forces for bacterial community composition in the endosphere and rhizosphere, whereas in bulk soil, all characteristics were strong determinants of community structure. Using nitrate and ammonium as constraints while excluding the other parameters explained 16% of the variation in bacterial community structure of the root endosphere (**Figure 3B**, PERMANOVA; $p < 0.05$). In the rhizosphere, 20% could be explained and in bulk soil 28% of variation in microbial community structure could be explained by soil ammonium and nitrogen (PERMANOVA; $p < 0.001$).

DISCUSSION

Microbial Diversity in Different Soil and Plant Compartments

The phylogenetic classification of plant-associated bacterial communities of *D. glomerata* highlighted *Proteobacteria* as the dominant phylum in all compartments, whereas other phyla including *Acidobacteria* and *Actinobacteria* were less present. *Proteobacteria* were found to be the predominating phylum across several plant species, including *Arabidopsis*, *Lotus japonicus*, *Hordeum vulgare* and *Agave* ssp. (Bulgarelli et al., 2012, 2015; Coleman-Derr et al., 2016; Zgadzaj et al., 2016). However, our data are only partly consistent with other studies performed in the same area. Kaiser et al. (2016) reported that beside *Proteobacteria*, *Acidobacteria* and *Actinobacteria* are highly abundant in soils from the "Schwäbische Alb." Similar results were obtained in other studies as well (Foesel et al., 2014). However, in both studies samplings were performed during spring, whereas our sampling took place in early summer. Accordingly, this change might be due to seasonal shifts in resource availability, which is indirectly caused by the plant composition through C allocation or nutrient uptake (Koranda et al., 2013). Furthermore, as the microbiome in bulk soil appeared to be fairly similar to rhizosphere, it is likely that the sampled "bulk soil" in our study has been strongly influenced by the surrounding plants.

Moreover, our results revealed that *Pseudomonadaceae* are highly abundant in the root endosphere, which is in line with

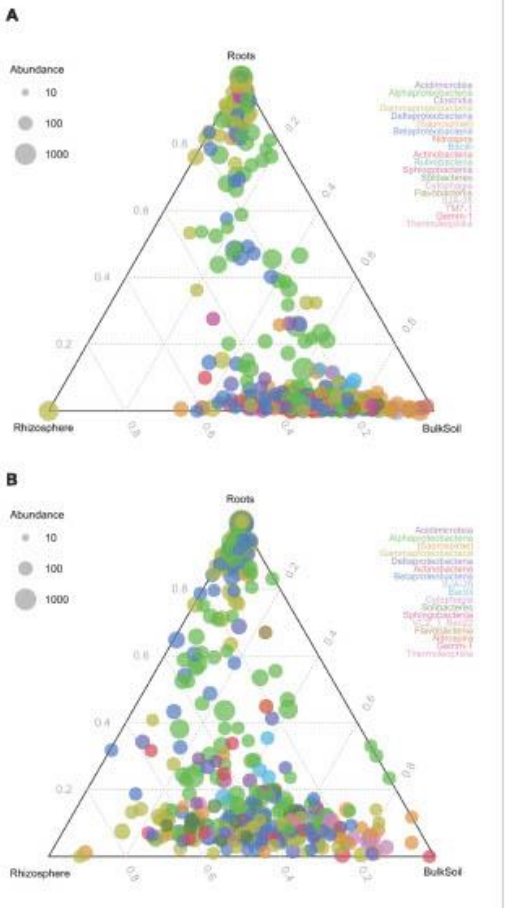

FIGURE 2 | Ternary plots of community structure in roots, rhizosphere and bulk soil. The colors represent the classes to which corresponding OTUs were assigned. **(A)** Represents distribution under high LUI, **(B)** under low LUI. The size of the plotted dots corresponds to the abundance of the OTUs with respect to each compartment. The position of each circle is determined by the contribution of the indicated compartments to the total abundance.

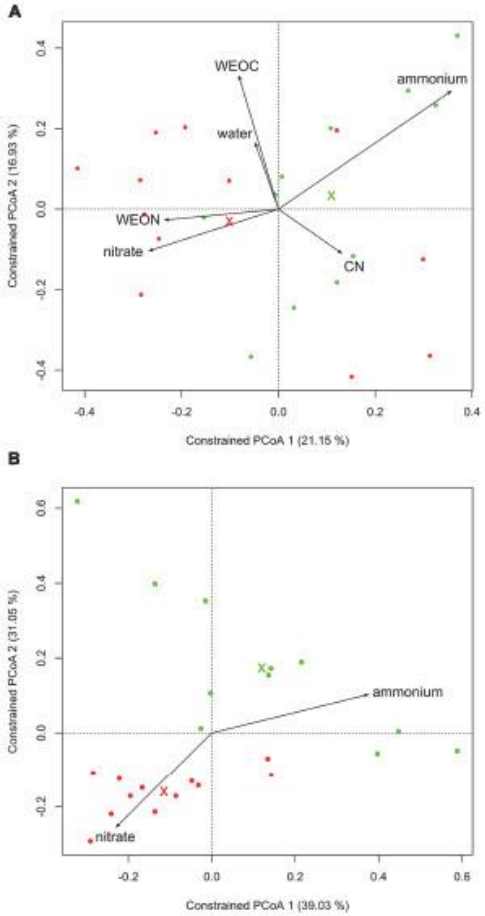

FIGURE 3 | Canonical analysis of principal coordinates of Bray–Curtis dissimilarities in roots. Crosses mark centroids of low or high LUI, respectively. Low LUI is represented in green, high LUI is represented in red color. **(A)** Using soil characteristics as constraints. Constraints reflect 36% of overall variance in the data (PERMANOVA, $p < 0.003$; 95% confidence interval), **(B)** using soil nitrate and ammonium as constrains independent from other soil characteristics. Constraints reflect 16% of overall variance in the data (PERMANOVA, $p < 0.019$; 95% confidence interval).

other studies investigating plant colonizing bacteria (Wemheuer et al., 2017). *Pseudomonadaceae* are reported to exhibit a wide range of plant-beneficial properties and plant growth-promoting traits. These include phosphate solubilization, nitrogen fixation, synthesis of cytokinins as well as of the phytohormones indole-3-acetic acid (IAA) and gibberellins (Cabanas et al., 2014; Vacheron et al., 2016). The latter was reported to affect seed formation and ripening in grasses (Stoddart, 1965; Kim et al., 2016), emphasizing the importance of *Pseudomonadaceae* in our study. The other abundant phyla detected in roots were *Rhizobiaceae*. Microbes of this family are known for antifungal properties as well as for plant growth-promoting, phosphate solubilizing and nitrogen-fixing abilities (Haack et al., 2016). Several members of *Oxalobacteraceae*, as well as *Enterobacteriaceae* and *Comamonadaceae* are frequently reported to be found inside roots and to possess plant-growth promoting effects (Bulgarelli et al., 2013; Taghavi et al., 2015; Cope-Selby et al., 2017). In contrast to our study, Wemheuer et al. (2017) described *Massilia*, which is a genus of *Oxalobacteraceae*, as the most abundant root endophyte in *D. glomerata*. Even though *Oxalobacteraceae*

were highly abundant root endophytes in our study, we could not detect the genus *Massilia* within our samples. To examine this in detail with careful and critical attention, further studies on the genus level have to be performed. Whereas Wemheuer et al. (2017) took their samples in autumn, when plants have already reached a senescence state, in our study samples were obtained during early summer, in the reproductive phase of the plant. Various studies have shown that both, land use and sampling season, influence the plant phenotype which in turn changes microbial patterns (Weaver, 1946; Bevivino et al., 2014). While this might explain the difference of abundant OTUs in both studies, another possible explanation for this incongruence might be the influence of various parameters like grazing, soil type, texture and chemical properties on bacterial community composition. Indeed, the latter appears to be highly probable since this genus was not found in other studies conducted at the same site (Kaiser et al., 2016).

Influence of Land Use Intensity on Plant-Associated Microbiomes

In this study, the main aim was to identify the impact of LUI on the plant-associated microbiome of *D. glomerata* during its reproductive stage. Interestingly, an increase of α-diversity was observed under intensive land use in bulk soil, which is in contrast to our initial hypothesis. This might be explained by an uneven distribution of certain bacterial taxa under high LUI, as it has been postulated that composition of microbial communities in intensively managed habitats tend to be highly variable (Garbeva et al., 2008). This in turn might lead to a transient and patchy colonization of the roots (Gottel et al., 2011). In contrast, microbial community composition in natural habitats with low human impact is considered to be more stable and thus colonization is more uniform (Bevivino et al., 2014). In this study, there was no influence of LUI on the microbial diversity in the rhizosphere. This corresponds to numerous studies that showed that rhizosphere microbial communities are more strongly affected by plant species rather than soil characteristics (Grayston et al., 1998; Gottel et al., 2011). However, a significant effect of LUI on the diversity was found in the roots. Analysis of differences in microbial communities revealed significant changes in qualitative measures (unweighted Unifrac) between high and low LUI in all compartments. These findings indicate that the divergence of the root and rhizosphere colonizing microbiota originates from the presence/absence of single OTUs across different LUIs, rather than on differences in the abundances of OTUs from different phyla. Significance in roots is therefore attributed to low-abundance OTUs, which are present at sites under low LUI or high LUI, respectively. However, most research has focused on the dominant community members rather than on less abundant phyla in the past. Still, abundance is not intimately connected with high metabolic activity (Hunt et al., 2013). Instead, the less abundant phylotypes might be metabolically more active or specific, thus strongly influencing the functional potential of microbial communities (Jousset et al., 2017). Hence, further investigations on the activity of microbial communities and their metabolic activities are required.

Nevertheless, results of diversity analysis corroborate our findings on the significant differences of the abundant bacterial families in roots. Most abundant taxa in the endosphere were not affected by LUI. It has been proposed that host tissues represent unique niches for associated bacteria, leading to little variation in dominant communities (Gottel et al., 2011). Only four families were significantly affected by LUI: *Turicibacteraceae*, *Bryobacteraceae*, *Cytophagaceae* and unassigned members of the *Rhizobiales*, albeit all of them were found in very low abundances (each less than 1% across all samples). Numerous families within *Rhizobiales*, e.g., *Rhizobiaceae*, are known for their plant-growth promoting and nitrogen-fixing abilities in *Fabaceae* (Patriarca et al., 2002; Chi et al., 2005). However, members of *Rhizobiales* were also found to express *nifH* in roots of sugarcane, a gene which encodes enzymes involved in biological nitrogen fixation (Rouws et al., 2014), thus enhancing plant performance under limited nitrogen availability. These findings indicate that *Rhizobiales* also play an important role in perennial grasses, albeit without nodule formation. Added to this, families of *Bryobacteraceae* were characterized as chemoheterotrophs that utilize various sugars and polysaccharides (Dedysh et al., 2016) and strains of *Cytophagaceae* are often reported to be able to fix nitrogen, digest polysaccharides or proteins as well as to utilize cellulose, making these (especially under low LUI) important for recycling of the most abundant carbohydrates produced by plants. Species belonging to these families have already been isolated from rhizosphere as well as plant tissues (McBride et al., 2014; Xu et al., 2014). In addition, *Turicibacteraceae* were mostly found across high LUIs. Species of this family are commonly found in the guts of animals (Auchtung et al., 2016), which could have been transferred through fertilization with manure or grazing by cattle in intensively managed sites. As a consequence, they were not further characterized as a plant growth-promoting taxon in other studies. However, *Turicibacteraceae* apparently are able to enter the endosphere of *D. glomerata* without causing visible disease symptoms. The frequency of these less abundant taxa suggests that they are sporadically acquired from the immediate environment, since they were not present in the core microbiome (Santhanam et al., 2015).

Together with the analysis of microbial patterns between the compartments (**Figure 2**), the results of the present study suggest the existence of a distinct microbial community in roots compared to rhizosphere and soil under both LUIs. Interestingly, a strong shift of community structure was observed from high to low LUI, leading to remarkably higher amounts of shared OTUs in samples from sites under low LUIs. Among OTUs uniquely found to be shared in all compartments under low LUI are OTUs which show close similarity to *Mesorhizobium*, which is well-known for its nitrogen-fixing and plant growth-promoting functions (Laranjo et al., 2014); this suggests that the plant recruits its microbiome from its surrounding soil in response to lower nutrient availability. Interaction between the plant and soil therefore seems to be more pronounced and specific under extensive land use.

Previous studies had indicated the strong influence of plants on the microbial colonization in the rhizosphere through exudation. However, it remained unclear whether soil type

or selection by the host plant was the major determinant of root microbiome composition (Girvan et al., 2003; Berg and Smalla, 2009; Berg et al., 2014). Since the soil type was identical in the plots of our study, compartments revealed 26% of variation (Bray–Curtis) in the data, whereas the soil properties nitrate and ammonium together explained only 16% of overall variation in roots. Based on these results we suggest an indirect contribution of nitrogen availability on the colonization of microbial communities in the plant. Recent studies have suggested that plants are able to sense their external and internal nitrogen status, demonstrating their ability to monitor and respond to changes to external nitrogen as well as endogenous nitrogen availability (Gent and Forde, 2017). Under low LUI, less nitrogen is available for the plant (see C/N ratio and soil characteristics in Supplementary Table S1 and Figure S1). Thus, the plants need to adjust to this persisting lower nitrogen status, which in turn might lead to change in exudation to influence its surrounding environment to their benefit (Lareen et al., 2016). In contrast, under high land use intensity, a high amount of nitrogen is introduced through fertilization, making specific attraction less important. Hence, land use intensity has an influence on the plant-specific traits that shape the plant–soil interface and thus indirectly influences the root microbial community.

CONCLUSION AND OUTLOOK

Our results indicate that roots of reproducing *D. glomerata* plants exhibit a unique niche for associated bacteria, which leads to a distinctive microbiome independent of land use intensity. Only rare root inhabiting taxa were influenced by LUI. We assume an indirect influence of LUI on the plant-associated microbiome as we observed a strong shift of community structure on the plant–soil interface. However, as abundance might not directly inform on metabolic activity, further more process oriented investigations are required addressing the functional potential of those microbial communities. Additionally, analysis of microbial composition in roots of *D. glomerata* in different plant developmental stages as well as root exudation may enhance knowledge on the dynamics of establishment of specific microbiomes at the plant–soil interface in response to land use intensity.

AUTHOR CONTRIBUTIONS

JE designed the experiment, carried out the field work, laboratory experiments, data analysis and wrote the manuscript. BS and PS contributed to the design of the experiment. PH contributed to the field work and the laboratory experiments. GV provided the pipelines for sequencing data analysis and advised data analysis. BS, PS, GV, JJ, MR, and MS advised the studies and critically revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2017.00930/full#supplementary-material

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

Supplemental Information Manuscript 1

Supplementary Material

The Influence of Land Use Intensity on the Plant Associated Microbiome of *Dactylis glomerata* L.

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1 Supplementary Figures and Tables

1.1 Supplementary Figures

Figure S1: Rarefaction curve of phylogenetic diversity in all compartments under low and high LUI at 97% sequence similarity. Bulk soil is depicted in blue, rhizosphere in red and root endosphere in orange. Lighter colors indicate low LUI.

Figure S2: Principal coordinate analysis (PCoA) based on dissimilarity matrices (95% confidence intervals). (A) unweighted Unifrac (R^2= 0.33, p<0.001), **(B)** weighted Unifrac (R^2= 0.33, p<0.001), **(C)** Bray-Curtis (R^2= 0.26, p<0.001).

Figure S3: VennDiagram of shared OTUs between all compartments in high versus low LUI. Red circle depicts high LUI, green circle depicts low LUI. Percentages refer to the total data.

1.2 Supplementary Tables

Table S1: Summary of edaphic parameters. Water extractable carbon/nitrogen (WEOC/WEON), nitrate, ammonium, C/N ratio in plants, gravimetric water content in the soil (water), Index: land use intensity index, calculated for 2006-2014, as described in Blüthgen et al. (2012) for grassland samples, the management and treatment of sampled sites.

Table S2: Relative abundance of assigned taxa. Groups that were less abundant than 1% in all compartments were grouped together in "Others". (A) most abundant phyla (B) most abundant families

Table S3: The impact of LUI on the β-diversity (between sample diversity) of bacterial community compositions. Significance (ADONIS, p-value < 0.05) indicated in bold letters, marginal significance (ADONIS, p-value < 0.1) indicated in italics.

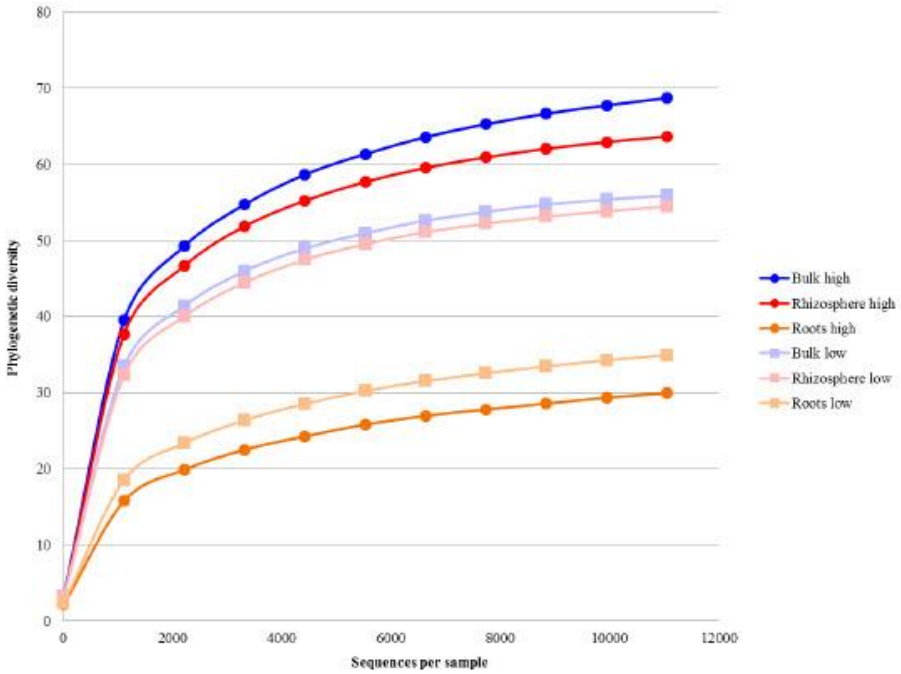
Table S4: Classification of shared core OTUs that were found in 95% of all samples.

Table S5: Classification of core OTUs that were found in 95% of all samples only under low land use intensities.

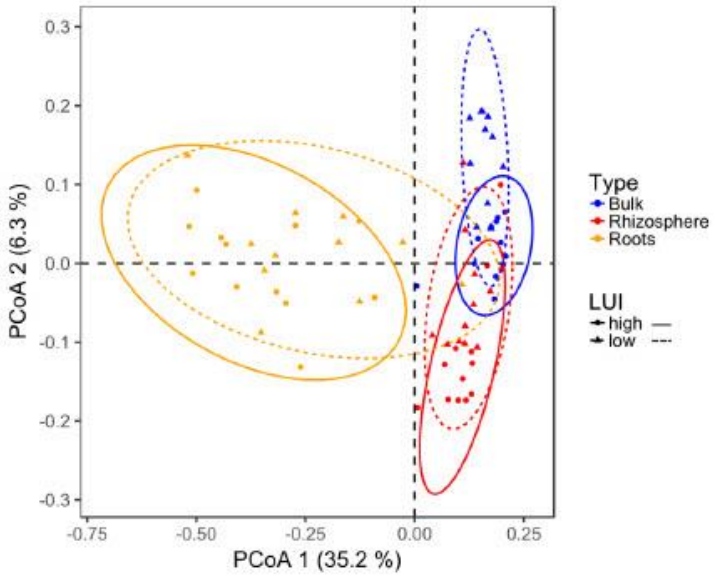
Table S6: Classification of core OTUs that were found in 95% of all samples only under high land use intensities.

Table S7: Families that are significantly affected by land use intensity. Significance was tested using t-test. Marginal significance ($p < 0.1$) is indicated in italics **(A)** in roots, **(B)** in rhizosphere and **(C)** in bulk soil

Figure S1


Phylogenetic diversity
Sequences per sample
0
10
20
30
40
50
60
70
80
0
2000
4000
6000
8000
10000
12000
Bulk high
Rhizosphere high
Roots high
Bulk low
Rhizosphere low
Roots low

57 **Figure S2A**



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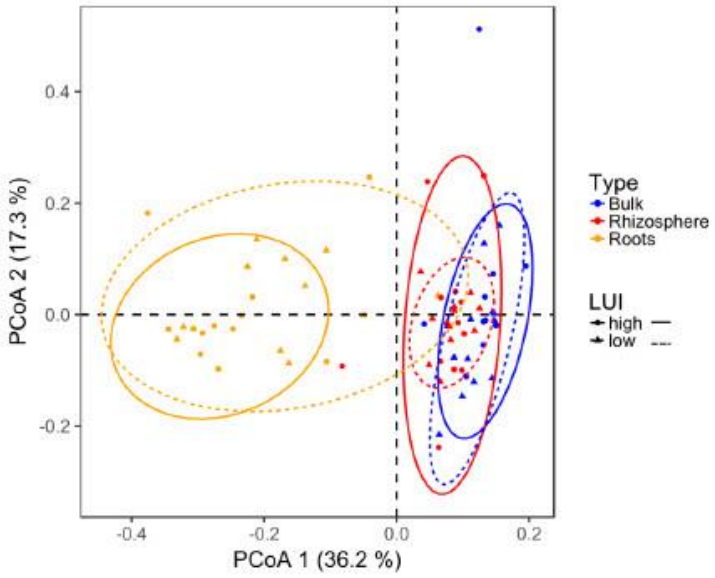
68 **Figure 2B**



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79 **Figure 2C**

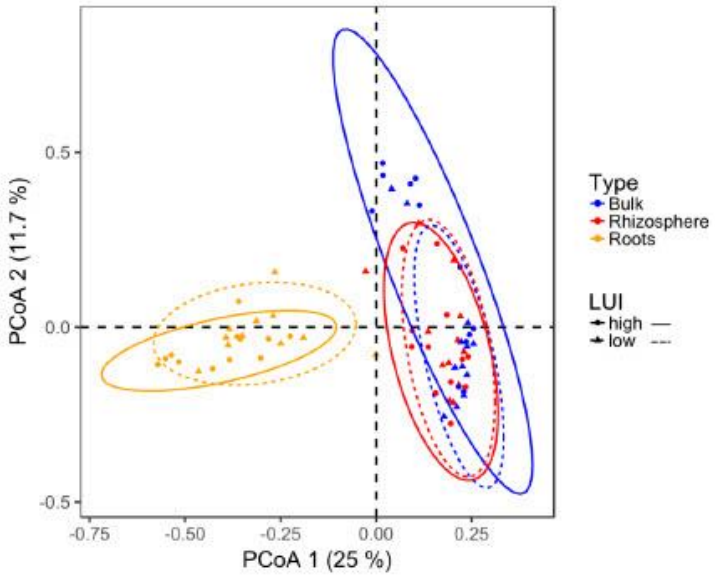

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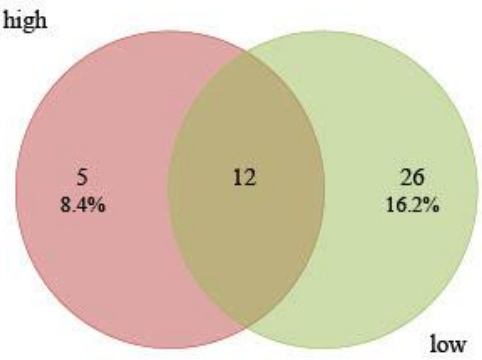
Figure S3



109 **Table S1**

Plot ID	WEOC [µg g-1 dw]	WEON [µg g-1 dw]	Nitrate [µg N g-1 dw]	Ammonium [µg N g-1 dw]	C/N ratio plants	Water (%)	Index (2006-2014)	Management	Treatment
AEG6	25.84±5.55	40.65±8.23	42.29±4.53	0.1±0.04	19.73±2.78	37	2.25	mown pasture	fertilized
AEG19	30.25±7.8	41.87±9.41	45.57±9.13	0.11±0.03	12.25±0.19	34	2.28	mown pasture	fertilized
AEG20	24.75±4.76	48.42±11.92	51.04±7.26	0.11±0.02	13.04±1.41	44	1.55	pasture	fertilized
AEG21	25.56±4.5	41.57±12.2	45.43±11.04	0.13±0.06	14.51±1.39	36	3.62	pasture	fertilized
AEG7	27.54±21.35	25.61±5.79	17.03±4.87	5.76±1.57	16.65±0.21	40	0.56	pasture	non-fertilized
AEG28	25.08±6.02	14.46±3.8	12.11±2.93	2.28±0.49	25.78±1.85	34	1.06	pasture	non-fertilized
AEG33	25.48±8.08	8.67±1.56	4.55±2.48	2.81±0.91	26.46±1.27	31	1.32	pasture	non-fertilized
AEG34	24.05±4.71	21.25±4.59	24.77±5.19	0.79±0.38	28.33±0.28	30	1.31	pasture	non-fertilized

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113 **Table S2A**

Phylum	Bulk RA (%)	Rhizosphere RA (%)	Roots RA (%)
Proteobacteria	0.60	0.69	0.91
Bacteroidetes	0.20	0.13	0.05
Actinobacteria	0.09	0.10	0.02
Acidobacteria	0.04	0.04	0.01
Firmicutes	0.02	0.01	0.01
Nitrospirae	0.01	0.01	<0.01
TM7	0.01	<0.01	<0.01
Chlorobi	0.01	<0.01	<0.01
Gemmatimonadetes	0.01	0.01	<0.01
Others	0.01	<0.01	<0.01

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127 **Table S2B**

Family	Bulk RA (%)	Rhizosphere RA (%)	Roots RA (%)
Pseudomonadaceae	0.01	0.01	0.15
Enterobacteriaceae	<0.01	0.01	0.12
Comamonadaceae	0.04	0.07	0.10
Oxalobacteraceae	<0.01	0.01	0.09
Rhizobiaceae	<0.01	0.01	0.09
Sphingomonadaceae	0.02	0.03	0.07
Xanthomonadaceae	0.03	0.06	0.06
Bradyrhizobiaceae	0.04	0.05	0.05
Sinobacteraceae	0.09	0.09	0.04
Burkholderiaceae	<0.01	<0.01	0.03
Phyllobacteriaceae	0.01	0.01	0.02
Chitinophagaceae	0.12	0.07	0.02
Hyphomicrobiaceae	0.10	0.08	0.02
Sphingobacteriaceae	<0.01	0.01	0.02
Caulobacteraceae	<0.01	0.01	0.02
Rhodospirillaceae	0.02	0.02	0.01
Xanthobacteraceae	<0.01	<0.01	0.01
Haliangiaceae	0.02	0.01	0.01
Mycobacteriaceae	<0.01	0.01	<0.01
Flavobacteriaceae	0.01	0.01	<0.01
Solibacteraceae	0.02	0.02	<0.01
Propionibacteriaceae	0.01	0.01	<0.01
Cytophagaceae	0.02	0.01	<0.01
Bacillaceae	0.01	0.01	<0.01
Micromonosporaceae	<0.01	0.01	<0.01
C111	0.01	0.01	<0.01
Alcaligenaceae	0.01	0.01	<0.01
EB1017	0.01	0.01	<0.01
Nitrospiraceae	0.01	0.01	<0.01
Gaiellaceae	0.01	0.01	<0.01
Syntrophobacteraceae	0.01	0.01	<0.01
Saprospiraceae	0.01	0.01	<0.01
Piscirickettsiaceae	0.01	<0.01	<0.01
Others	0.32	0.31	0.06

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131 **Table S3**

Compartment	Distance metrics	R^2	p-value
Roots	**Unweighted**	**0.08093**	**0.015**
	Weighted	0.05146	0.278
	Bray-Curtis	0.05291	0.16
Rhizosphere	**Unweighted**	**0.08949**	**0.003**
	Weighted	0.05793	0.202
	Bray-Curtis	*0.07037*	*0.068*
Bulk soil	**Unweighted**	**0.1441**	**0.001**
	Weighted	0.0747	0.124
	Bray-Curtis	**0.13575**	**0.008**

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Table S4

OTU ID	Kingdom	Phylum	Class	Order	Family	Genus
573764	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*
4377104	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*
1105814	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*
580625	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*
866365	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*
731707	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*
317632	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
573013	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*
646549	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
969805	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*
1003206	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
573258	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*

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Table S5

OTU ID	Kingdom	Phylum	Class	Order	Family	Genus
399818	Bacteria	Proteobacteria	Betaproteobacteria	Ellin6067	Not assigned	Not assigned
113261	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Not assigned	Not assigned
254098	Bacteria	Proteobacteria	Betaproteobacteria	Not assigned	Not assigned	Not assigned
838594	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
961922	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
580703	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*
573135	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*
358785	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
545247	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
616682	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
2025156	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
210914	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
New.0.CleanUp.ReferenceOTU4459	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Not assigned*
New.1.CleanUp.ReferenceOTU99	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*
620684	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*
547097	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*
571263	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*
398604	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
350105	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
3314521	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
1104627	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*
709657	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Not assigned
281360	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	Not assigned
4373617	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Not assigned
New.0.CleanUp.ReferenceOTU3078	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Not assigned
New.0.CleanUp.ReferenceOTU8268	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*

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Table S6

OTU ID	Kingdom	Phylum	Class	Order	Family	Genus
811449	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Not assigned
689696	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*
211171	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Not assigned
849118	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*
850572	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*

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Table S7A

Kingdom	Phylum	Class	Order	Family	p-value	RA high LUI (%)	RA low LUI (%)
Bacteria	**Firmicutes**	**Bacilli**	**Turicibacterales**	**Turicibacteraceae**	**0.022**	**0.018**	**0.002**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhizobiales**	**Other**	**0.030**	**0.049**	**0.182**
Bacteria	**Acidobacteria**	**Solibacteres**	**Solibacterales**	**[Bryobacteraceae]**	**0.033**	**0.016**	**0.093**
Bacteria	**Bacteroidetes**	**Cytophagia**	**Cytophagales**	**Cytophagaceae**	**0.035**	**0.194**	**0.395**
Bacteria	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*0.054*	0.003	0.035
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Not assigned	*0.060*	0.098	0.367
Bacteria	Firmicutes	Bacilli	Bacillales	Not assigned	*0.064*	0.019	0.052
Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*0.065*	1.978	7.375
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Actinosynnemataceae	*0.070*	0.030	0.001
Bacteria	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	*0.076*	0.002	0.012
Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	*0.077*	0.002	0.015
Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*0.080*	0.149	0.427
Bacteria	Bacteroidetes	VC2_1_Bac22	Not assigned	Not assigned	*0.083*	0.006	0.038
Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	*0.088*	0.034	0.000
Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	*0.092*	0.185	0.028
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	*0.098*	0.002	0.016
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Not assigned	*0.099*	0.337	0.808
Bacteria	Proteobacteria	Betaproteobacteria	SC-I-84	Not assigned	*0.099*	0.134	0.390

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Table S7B

Kingdom	Phylum	Class	Order	Family	p-value	RA high LUI (%)	RA low LUI (%)
Bacteria	**Proteobacteria**	**Deltaproteobacteria**	**Myxococcales**	**Cystobacteraceae**	**0.0003**	**0.095**	**0.205**
Bacteria	**Nitrospirae**	**Nitrospira**	**Nitrospirales**	**Nitrospiraceae**	**0.0003**	**1.356**	**0.200**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Not assigned**	**Not assigned**	**0.0010**	**0.082**	**0.257**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhizobiales**	**Not assigned**	**0.0022**	**0.648**	**0.953**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhodospirillales**	**Rhodospirillaceae**	**0.0022**	**1.712**	**2.384**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**Ellin6067**	**Not assigned**	**0.0028**	**2.966**	**4.321**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**Nitrosomonadales**	**Nitrosomonadaceae**	**0.0042**	**0.082**	**0.007**
Bacteria	**Proteobacteria**	**TA18**	**PHOS-HD29**	**Not assigned**	**0.0042**	**0.012**	**0.003**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Ellin329**	**Not assigned**	**0.0050**	**0.307**	**0.617**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Hyphomonadaceae**	**0.0062**	**0.237**	**0.417**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhodobacterales**	**Rhodobacteraceae**	**0.0066**	**0.351**	**0.159**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**IS-44**	**Not assigned**	**0.0227**	**0.074**	**0.246**
Bacteria	**Actinobacteria**	**Actinobacteria**	**Actinomycetales**	**Propionibacteriaceae**	**0.0230**	**1.206**	**0.470**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**Rhodocyclales**	**Rhodocyclaceae**	**0.0244**	**0.314**	**0.133**
Bacteria	**Tenericutes**	**Mollicutes**	**Anaeroplasmatales**	**Anaeroplasmataceae**	**0.0334**	**0.001**	**0.010**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhizobiales**	**Beijerinckiaceae**	**0.0356**	**0.038**	**0.022**
Bacteria	**Acidobacteria**	**Acidobacteriia**	**Acidobacteriales**	**Acidobacteriaceae**	**0.0358**	**0.002**	**0.012**
Bacteria	**Actinobacteria**	**Actinobacteria**	**Actinomycetales**	**Intrasporangiaceae**	**0.0440**	**0.047**	**0.020**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rickettsiales**	**Not assigned**	**0.0461**	**0.015**	**0.026**
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*0.0532*	0.124	0.049
Bacteria	Proteobacteria	Gammaproteobacteria	Chromatiales	Not assigned	*0.0550*	0.002	0.010
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Pseudonocardiaceae	*0.0567*	0.434	0.200
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Not assigned	*0.0575*	0.182	0.083
Bacteria	Proteobacteria	Gammaproteobacteria	Other	Other	*0.0580*	0.119	0.231
Bacteria	Acidobacteria	Solibacteres	Solibacterales	Not assigned	*0.0630*	1.054	1.427
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	*0.0747*	0.012	0.043
Bacteria	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	*0.0753*	0.251	0.528
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*0.0801*	1.223	0.509
Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*0.0812*	0.554	1.363
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Frankiaceae	*0.0815*	0.184	0.054
Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	*0.0833*	0.117	0.049

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Bacteria	Acidobacteria	DA052	Ellin6513	Not assigned	*0.0873*	0.000	0.020
Bacteria	Chloroflexi	Anaerolineae	SHA-20	Not assigned	*0.0875*	0.002	0.012
Bacteria	Proteobacteria	Betaproteobacteria	Not assigned	Not assigned	*0.0918*	1.575	2.388
Bacteria	Proteobacteria	Deltaproteobacteria	Not assigned	Not assigned	*0.0941*	0.019	0.049
Bacteria	Proteobacteria	Gammaproteobacteria	[Marinicellales]	[Marinicellaceae]	*0.0960*	0.073	0.301
Bacteria	TM6	SJA-4	Not assigned	Not assigned	*0.0985*	0.025	0.107
Bacteria	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*0.0992*	0.175	0.421

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Supplementary Material

Table S7C

Kingdom	Phylum	Class	Order	Family	p-value	RA high LUI (%)	RA low LUI(%)
Bacteria	Proteobacteria	Betaproteobacteria	A21b	UD5	0.0002	0.422	0.167
Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	0.0002	0.366	0.015
Bacteria	Proteobacteria	Alphaproteobacteria	Ellin329	Not assigned	0.0002	0.200	0.401
Bacteria	Elusimicrobia	Elusimicrobia	FAC88	Not assigned	0.0003	0.024	0.004
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	0.0003	0.141	0.034
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Intrasporangiaceae	0.0013	0.031	0.003
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	125ds10	0.0015	0.027	0.077
Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	0.0016	1.395	0.212
Bacteria	OD1	ABY1	Not assigned	Not assigned	0.0016	0.011	0.000
Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	0.0020	1.528	2.585
Bacteria	Proteobacteria	Betaproteobacteria	Nitrosomonadales	Nitrosomonadaceae	0.0022	0.121	0.005
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Not assigned	0.0025	0.710	0.975
Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	211ds20	0.0033	0.032	0.005
Bacteria	Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae	0.0050	0.034	0.006
Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	0.0053	0.285	0.082
Bacteria	Chloroflexi	Ellin6529	Not assigned	Not assigned	0.0056	0.046	0.019
Bacteria	Actinobacteria	MB-A2-108	0319-7L14	Not assigned	0.0063	0.065	0.018
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micromonosporaceae	0.0072	0.228	0.507
Bacteria	Proteobacteria	Deltaproteobacteria	NB1-j	NB1-i	0.0073	0.168	0.417
Bacteria	Planctomycetes	C6	d113	Not assigned	0.0074	0.011	0.000
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Not assigned	0.0106	2.976	4.215
Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Not assigned	0.0109	0.009	0.000
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Streptosporangiaceae	0.0123	0.011	0.111
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	0.0160	0.832	0.417

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Bacteria	**Proteobacteria**	**Deltaproteobacteria**	**Myxococcales**	**Cystobacteraceae**	**0.0175**	**0.100**	**0.280**
Bacteria	**Nitrospirae**	**Nitrospira**	**Nitrospirales**	**FW**	**0.0187**	**0.044**	**0.000**
Bacteria	**Proteobacteria**	**Deltaproteobacteria**	**Spirobacillales**	**Not assigned**	**0.0210**	**0.008**	**0.001**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhizobiales**	**Xanthobacteraceae**	**0.0215**	**0.064**	**0.103**
Bacteria	**TM6**	**SJA-4**	**Not assigned**	**Not assigned**	**0.0225**	**0.090**	**0.308**
Bacteria	**Planctomycetes**	**Other**	**Other**	**Other**	**0.0229**	**0.014**	**0.047**
Bacteria	**Actinobacteria**	**Thermoleophilia**	**Gaiellales**	**Gaiellaceae**	**0.0243**	**1.687**	**1.000**
Bacteria	**Acidobacteria**	**Solibacteres**	**Solibacterales**	**AKIW659**	**0.0263**	**0.048**	**0.012**
Bacteria	**Gemmatimonadetes**	**Gemmatimonadetes**	**Ellin5290**	**Not assigned**	**0.0279**	**0.041**	**0.195**
Bacteria	**Proteobacteria**	**Deltaproteobacteria**	**Myxococcales**	**Polyangiaceae**	**0.0291**	**0.252**	**0.353**
Bacteria	**Spirochaetes**	**[Leptospirae]**	**[Leptospirales]**	**Leptospiraceae**	**0.0295**	**0.056**	**0.030**
Bacteria	**Bacteroidetes**	**Sphingobacteriia**	**Sphingobacteriales**	**Sphingobacteriaceae**	**0.0301**	**0.204**	**0.064**
Bacteria	**Actinobacteria**	**Actinobacteria**	**Actinomycetales**	**Pseudonocardiaceae**	**0.0324**	**0.281**	**2.107**
Bacteria	**Chloroflexi**	**TK17**	**mle1-48**	**Not assigned**	**0.0354**	**0.019**	**0.006**
Bacteria	**Acidobacteria**	**EC1113**	**Not assigned**	**Not assigned**	**0.0357**	**0.014**	**0.005**
Bacteria	**Gemmatimonadetes**	**Gemmatimonadetes**	**Gemmatimonadales**	**Ellin5301**	**0.0378**	**0.006**	**0.098**
Bacteria	**Proteobacteria**	**Alphaproteobacteria**	**Rhizobiales**	**Beijerinckiaceae**	**0.0389**	**0.051**	**0.032**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**Rhodocyclales**	**Rhodocyclaceae**	**0.0403**	**0.336**	**0.092**
Bacteria	**WS3**	**PRR-12**	**Sediment-1**	**Not assigned**	**0.0408**	**0.036**	**0.010**
Bacteria	**Actinobacteria**	**Acidimicrobiia**	**Acidimicrobiales**	**AKIW874**	**0.0429**	**0.366**	**0.774**
Bacteria	**Actinobacteria**	**Actinobacteria**	**Actinomycetales**	**Other**	**0.0432**	**0.133**	**0.227**
Bacteria	**Firmicutes**	**Bacilli**	**Turicibacterales**	**Turicibacteraceae**	**0.0448**	**0.021**	**0.005**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**Burkholderiales**	**Oxalobacteraceae**	**0.0455**	**0.648**	**0.267**
Bacteria	**Proteobacteria**	**Betaproteobacteria**	**Methylophilales**	**Methylophilaceae**	**0.0487**	**0.167**	**0.036**
Bacteria	Chlorobi	OPB56	Not assigned	Not assigned	*0.0502*	0.024	0.009
Bacteria	Planctomycetes	Not assigned	Not assigned	Not assigned	*0.0503*	0.032	0.064
Bacteria	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	*0.0526*	0.008	0.021
Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Not assigned	*0.0536*	2.927	1.546

Bacteria	Acidobacteria	Solibacteres	Solibacterales	Not assigned	*0.0580*	1.353	2.139
Bacteria	Chloroflexi	Anaerolineae	SBR1031	A4b	*0.0611*	0.034	0.082
Bacteria	Chlamydiae	Chlamydiia	Chlamydiales	Simkaniaceae	*0.0645*	0.006	0.031
Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	*0.0680*	0.027	0.015
Bacteria	Acidobacteria	DA052	Ellin6513	Not assigned	*0.0693*	0.002	0.023
Bacteria	WS3	PRR-12	LD1-PA13	Not assigned	*0.0696*	0.046	0.143
Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*0.0785*	0.194	0.104
Bacteria	WS3	PRR-12	Sediment-1	PRR-10	*0.0799*	0.003	0.011
Bacteria	Proteobacteria	Betaproteobacteria	SC-I-84	Not assigned	*0.0818*	2.586	3.524
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	*0.0831*	0.020	0.000
Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*0.0844*	1.598	2.384
Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Nannocystaceae	*0.0851*	0.077	0.036
Bacteria	GN02	3BR-5F	Not assigned	Not assigned	*0.0857*	0.009	0.000
Bacteria	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*0.0878*	0.366	0.597
Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	*0.0896*	0.070	0.038
Bacteria	Gemmatimonadetes	Gemm-5	Not assigned	Not assigned	*0.0916*	0.008	0.031
Bacteria	Chlorobi	SJA-28	Not assigned	Not assigned	*0.0932*	0.871	0.284
Bacteria	Proteobacteria	Betaproteobacteria	Other	Other	*0.0998*	0.046	0.024

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Definition of Core Bacterial Taxa in Different Root Compartments of *Dactylis glomerata*, Grown in Soil under Different Levels of Land Use Intensity

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Abstract: Plant-associated bacterial assemblages are critical for plant fitness. Thus, identifying a consistent plant-associated core microbiome is important for predicting community responses to environmental changes. Our target was to identify the core bacterial microbiome of orchard grass *Dactylis glomerata* L. and to assess the part that is most sensitive to land management. *Dactylis glomerata* L. samples were collected from grassland sites with contrasting land use intensities but comparable soil properties at three different timepoints. To assess the plant-associated bacterial community structure in the compartments rhizosphere, bulk soil and endosphere, a molecular barcoding approach based on high throughput 16S rRNA amplicon sequencing was used. A distinct composition of plant-associated core bacterial communities independent of land use intensity was identified. *Pseudomonas*, *Rhizobium* and *Bradyrhizobium* were ubiquitously found in the root bacterial core microbiome. In the rhizosphere, the majority of assigned genera were *Rhodoplanes*, *Methylibium*, *Kaistobacter* and *Bradyrhizobium*. Due to the frequent occurrence of plant-promoting abilities in the genera found in the plant-associated core bacterial communities, our study helps to identify "healthy" plant-associated bacterial core communities. The variable part of the plant-associated microbiome, represented by the fluctuation of taxa at the different sampling timepoints, was increased under low land use intensity. This higher compositional variation in samples from plots with low land use intensity indicates a more selective recruitment of bacteria with traits required at different timepoints of plant development compared to samples from plots with high land use intensity.

Keywords: land use intensity; plant-associated microbiome; endophytes; rhizosphere; biodiversity; bacteria; core microbiome; *Pseudomonas*

1. Introduction

It is generally accepted that microbiomes support plant growth and health at the plant soil interface [1–4]. The soil influenced by the root (rhizosphere) and the plant inner tissue (endosphere) provides distinct habitats for these microbial communities. In the rhizosphere, microorganisms benefit from exudation of organic compounds by the plant as well as mucilage provides ecological niches for important plant growth promoting microorganisms [5]. Moreover, plants secrete compounds to selectively chemoattract microorganisms, facilitating their colonization of and proliferation in the

rhizosphere [6]. Endophytic bacteria are usually recruited from the rhizosphere microbiome and enter the plant either through lesions or penetrate the root surface actively in addition to vertical transmission of microbes via seeds [7,8]. Despite strong seasonal variations in relation to the plant development stage, the structure and function of plant-associated microbiomes are mostly influenced by the plant species [9].

However, in addition to the plant, microbial communities at the plant–soil interface are also shaped by other factors, including abiotic site-specific properties like soil characteristics, water availability and temperature. Furthermore, the type of management of a particular site and its intensity have been considered important drivers of the plant-associated microbial community [3,10–14]. We could recently show for *Dactylis glomerata* L., grown in a number of different grassland soils across Germany, that, independent of land use intensity (LUI), members of *Pseudomonadaceae*, *Enterobacteriaceae* and *Comamonadaceae* were the most abundant root endophytes, whereas in the rhizosphere and bulk soil, a clear influence of LUI on the microbial community structure was evident [15]. However, only a single date during peak vegetation was taken into account in this study, which might have masked effects of land use intensity on root endophytes due to the plants' impact as a result of high exudation rates at the selected sampling time.

Numerous studies postulate that despite the dynamic nature of the plant-associated microbial communities, plants may harbor a species-specific set of essential microbes that are not influenced by environmental conditions and plant growth, supporting the concept of plant-associated core microbial communities [16,17]. Those members may be crucial for nutrient uptake as well as the stress response of the plant and consequently determine the overall fitness of a plant [18]. Thus, the loss of parts of the plant-associated core microbial communities may induce reduced plant fitness and in the long run out-competition by other plants, which triggers significant shifts in biodiversity pattern worldwide, mainly if the plants are facing abiotic and biotic stressors. However, the consequences of LUI for the formation of a particular plant-associated core microbial composition at the root–soil interface are so far unclear.

In the frame of this study we selected eight grassland sites with different LUI within the Biosphere Reserve "Schwäbische Alb" in Southwestern Germany, to identify and define a putative root-associated bacterial core microbiome in (a) the endophytic compartment and (b) the rhizosphere of *D. glomerata*. Though plant-associated microbes comprise also of different entities such as fungi or protists, we focused in our study on plant-associated bacterial communities for which highly standardized analytical pipelines have been developed in the last years. For all sites, we analyzed bacterial diversity pattern using a molecular barcoding approach at different plant growth stages. We postulated that intensive land use (high LUI) may disentangle the close association of plants and microbes in terms of their co-occurrence and the composition of the core bacterial communities at the plant–soil interface (rhizosphere) might be less complex in terms of reduced bacterial diversity compared to sites with extensive forms of land use (low LUI). We expected that this effect be more pronounced in the rhizosphere than in the root interior.

2. Materials and Methods

2.1. Sampling Sites

The present study was conducted within the long-term interdisciplinary project of the German "Biodiversity Exploratories" (http://www.biodiversity-exploratories.de). Our study sites were located at the "Schwäbische Alb", which is a limestone secondary mountain range in the southwest of Germany, covering an area of about 422 km^2. The land use intensity of the experimental plots was assessed by a land use intensity index according to Blüthgen et al. [19], which was calculated for consecutive years (2006–2014) and included the three major management components fertilization, livestock density and mowing frequency (Table S1). The intensities of the different components were normalized to the regional mean. The index reflects a numerical gradient, formed by calculating the sum of the

normalized LUI components equally weighted. For detailed equations please see Blüthgen et al. [19]. Plots were classified as high/low LUI based on their LUI index throughout several years (2006–2010). Selected sites were AEG6, AEG19, AEG20 and AEG21, representing high LUI plots and AEG7, AEG28, AEG33 and AEG34, representing low LUI plots (details can be seen in Table S1). Low LUI index ranged from 0.56 to 1.31 and high LUI index ranged from 1.55 to 2.25. The soil type of all plots was described as Rendzic Leptosol (according to the FAO classification system). Soil texture of the plots was determined as follows: clay content was in the range of 423–637 g/kg soil, silt content in the range of 327–554 g/kg soil and sand content in the range of 15–69 g/kg soil with exception of plot AEG7, showing differing values (clay: 385 g/kg soil, silt: 427 g/kg soil and sand: 188 g/kg soil). Total carbon contents of the sites were in a comparable range from 47 to 88 g/kg soil, total nitrogen contents were in the range of 4.8–10.6 g/kg soil. The C to N ratio was between 9 and 11 for all plots investigated. Samples were collected in May, June and October 2015, representing dynamic seasonal variations. The mean annual temperature was 9.9 °C and the annual precipitation was 730 mm. Mean temperature and precipitation during the months of sampling collection was 8.5–19 °C and 67.1 mm in May, 12.3–22.7 °C and 80.6 mm in June and 5.2–13.3 °C and 19.1 mm in October (Deutscher Wetterdienst, Offenbach, Station Stuttgart/Echterdingen). At each plot, samples were collected within a subplot of 1.5 m × 1.5 m. At each sampling timepoint, three plants of *D. glomerata* without any disease symptoms were excavated per plot and treated as true replicates.

2.2. Sampling and Basic Analyses

Roots of *D. glomerata* with adhering rhizosphere soil were suspended in 7.5 mL sterile 1× PBS solution amended with 0.02% Silwet (PBS, AppliChem, Darmstadt, Germany; Silwet L-77), and shaken at 180 rpm for 5 min to separate the rhizosphere from the roots. This step was repeated three times. The PBS solution containing the collected rhizosphere soil was centrifuged at 5000× *g* for 5 min and the pellet was frozen in liquid nitrogen and stored at −80 °C until further analyses.

After the separation of rhizosphere soil, roots were immediately surface sterilized. Therefore, roots were subjected to sterile 1% Tween 20 for 2 min and washed with pure autoclaved water. Next, roots were incubated for 2 min in 70% ethanol and rinsed three times in sterile distilled water. Subsequently, surface sterilization was done by incubating the roots in 5% sodium hypochlorite for 10 min and rinsing them in sterile water eight times. Sterilized roots were frozen in liquid nitrogen and stored at −80 °C. To check successful surface sterilization, DNA extraction followed by PCR amplification of the 16S rRNA genes, and incubation of 200 µL of the final rinse water on NB agar plates was performed. The absence of the PCR product and no colonies on the agar after 10 days at 28 °C confirmed successful sterilization.

Furthermore, bulk soil samples were taken (soil only loosely attached to the roots) and stored at −80 °C until further molecular analyses, respectively, sieved and stored for a maximum of 24 h at 4 °C for chemical analysis. Water extractable organic carbon (WEOC) and nitrogen (WEON) were determined using DIMA-TOC 100 (Dima Tec, Langenhagen, Germany) using 0.01 M calcium chloride solution for extraction [20]. The same extracts were used to measure nitrate (NO_3^--N) and ammonium (NH_4^+-N) by continuous flow analysis using a photometric autoanalyzer (CFA-SAN Plus; Skalar Analytik, Erkelenz, Germany).

In addition, above ground plant material was collected and rinsed with tap water, dried on 65 °C for 2 days and pulverized using the Tissue LyserII (Qiagen GmbH, Hilden, Germany). Total carbon and nitrogen contents were measured using an Elemental Analyzer 'Euro-EA' (Eurovector, Milano, Italy).

2.3. Nucleic Acid Extraction

For nucleic acid extraction, a phenol-chloroform-based method was used with slight modifications [21]. We used surface sterilized roots, the rhizosphere and bulk soil from all eight plots (3 replicates per plot) in May, June and October resulting in 216 samples in total. Prior to DNA extraction, surface sterilized roots were frozen in liquid nitrogen and prehomogenized using a

TissueLyserII (Qiagen GmbH, Hilden, Germany). Afterwards, 0.1 g of roots and 0.3 g of rhizosphere and bulk soil were homogenized using lysing matrix tubes E (MP Biomedicals, Illkirch-Graffenstaden, France) in 120 mM sodium phosphate buffer (pH 8) and TNS solution (500 mM Tris-HCl pH 8.0, 100 mM NaCl, 10% SDS (wt/vol)), and centrifuged at 16,100× *g* for 10 min at 4 °C. The supernatant was transferred into a 2 mL DNase/RNase free SafeLock tube on ice and successively mixed with an equal volume of phenol/chloroform/isoamylalcohol (25:24:1 (vol/vol), Sigma-Aldrich, St. Louis, MO, USA) and chloroform/isoamylalcohol (24:1 (vol/vol)) and centrifuged for 5 min at 16,100× *g*. DNA was precipitated using 30% (wt/vol) polyethylene glycol (PEG) solution (PEG 6000, NaCl). After 2 h of incubation on ice, the solution was centrifuged (16,100× *g*, 10 min, 4 °C). The resulting pellet was washed in ice-cold DNase/RNase free 70% ethanol, air-dried and eluted in 30 µL 0.1% diethylpyrocarbonate water. The concentration was measured in duplicates using the Quant-iT™Pico Green® ds DNA assay Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The measurements were performed with a SpectraMax Gemini EM Fluorescence Plate Reader Spectrometer (Molecular Devices, Sunnyvale, CA, USA). Values were corrected for background fluorescence by addition of negative controls. Finally, the DNA extracts were stored at −80 °C until further use.

2.4. Library Preparation and Illumina Sequencing

Next generation sequencing was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA). Library was prepared according to the "16S Metagenomic Sequencing Library Preparation" protocol proposed by Illumina Inc., USA. To reduce biases of the polymerase chain reaction (PCR) amplification of the 16S rRNA region, the reaction was carried out in triplicates using 335Fc (5′-CADACTCCTACGGGAGGC-3′) as a forward primer and 769Rc (5′-ATCCTGTTTGMTMCCCVCRC-3′) as a reverse primer with Illumina adapter sequences [22]. The PCR reaction contained 12.5 µL NEB Next High Fidelity Master Mix (Illumina Inc., San Diego, CA, USA), 0.5 µL of each primer (10 pmol/µL), 2.5 µL of 3% BSA, 100–200 ng of template DNA and 25 µL of DEPC water. PCR conditions included an initial denaturation step at 98 °C for 5 min, followed by 20 cycles (rhizosphere and bulk soil samples) or 28 cycles (root samples) of denaturation (98 °C; 10 s), annealing (60 °C; 30 s) and elongation (72 °C; 30 s). The final elongation was performed at 72 °C for 5 min. Negative controls of extraction (blank extraction) and PCR (using DEPC water instead of template DNA) were treated accordingly. Resulting amplicons were analyzed on a 2% agarose gel. Afterwards, triplicates were pooled and purified using the Agencourt®AMPure®XP (Beckman Coulter Company, Carlsbad, CA USA) extraction kit according to the manufacturer's instructions, with a modified ratio of AMPure XP to PCR reaction (0.6/1). The presence of primer-dimers and amplicon sizes were checked on a Bioanalyzer 2100 Agilent Technologies, Santa Clara, CA, USA), using the DNA 7500 kit (Agilent Technologies, Santa Clara, CA, USA) and quantified using the Quant-iT PicoGreen kit (Life Technologies, Grand Island, NY, USA). Finally, indexing PCR was carried out using 10 ng of amplicon DNA, 12.5 µL NEB Next High Fidelity Master Mix and 10 pmol of each indexing-primer. PCR conditions were changed for the annealing temperature (55 °C) and the number of cycles (8 cycles). Purified PCR products were pooled in equimolar ratios to a final concentration of 4 nM and sequenced using the MiSeq Reagent kit v3 (600 cycles; Illumina Inc., San Diego, CA, USA) for paired end sequencing. Sequence files were deposited in the NCBI Sequence Read Archive under accession numbers SRP102620 and PRJNA380810.

2.5. Sequence Data Analysis

Sequence analysis was performed using QIIME (quantitative insights into microbial ecology [23]) and default parameters. FASTQ files were trimmed and merged with a minimum read length of 50 and minimum Phred score of 15 using AdapterRemoval [24]. PhiX contamination was removed using DeconSeq [25]. Reads were merged and filtered by size (400–480 bp) and clustered into operational taxonomic units (OTUs) at 97% sequence identity with an open reference strategy using GreenGenes 16S rRNA reference database (13_5 release) [26]. Taxonomy was assigned using the RDP (v2.2) classifier

retrained on the GreenGenes [27]. Afterwards, chloroplast sequences were removed, and output was filtered with an abundance cut-off of 0.001%. To make results comparable, the data set was rarefied to the lowest obtained read number. Afterwards, diversity analyses were performed, which is implemented in the Qiime workpackages, including the calculation of relative abundance of each OTU per sample as well as the computation of α- and ß-diversity. The analysis of α-diversity (within sample diversity) was calculated per sample and was based on chao1 richness [28] and Shannon's diversity [29]. For the calculation of the boxplots, samples were grouped by month and LUI (i.e., 3 × 4 samples high and 3 × 4 samples low LUI per sampling season and compartment). Significant differences in α-diversity were obtained by unpaired *t*-tests. Betadiversity measures were calculated using unweighted and weighted UniFrac metrics as described by Catherine et al. [30].

Statistical significance of dissimilarities in ß-diversity was determined by adonis using the r-package, Vegan (R package version 2.4-4) [31], via the Qiime script "compare_categories.py". Significances of LUI were calculated per compartment and season. Significances of season were calculated per compartment and LUI. The analysis of the core bacterial microbiome of roots and rhizosphere from different LUIs was based on the total relative abundance of bacterial OTUs and was computed using "compute_core_microbiome.py". Thus, a table of OTUs was obtained per month and LUI, where the OTUs that remained were present in 90% of the respective samples (i.e., OTU had to be present in 11 of 12 samples per LUI and month). These tables were used for visualization in the Bioinformatics and Evolutionary Genomics webtool [32].

3. Results

3.1. Soil Carbon and Nitrogen Content

While WEOC was not influenced by different LUI levels, it changed in response to the sampling season. Concentrations in October (64.1 μg g^{-1} dw in average) were significantly higher compared to May (35 μg g^{-1} dw in average) or June (26.1 μg g^{-1} dw in average). In contrast, WEON, nitrate and ammonium concentrations increased with LUI and changed over time. As expected, the highest concentrations of 43.13 μg g^{-1} dw WEON and 46.08 μg N g^{-1} dw nitrate on average were detected in June on intensively managed sites due to increased fertilizer input by manure application (see Table S2).

3.2. Sequencing Summary

In total, 16,085,722 raw-sequence reads were obtained from PCR amplicons by Illumina sequencing. After quality filtering and chimera check, 12,561,385 high-quality partial 16S rRNA gene sequences with a minimum length of 400 bp remained. After removal of chloroplasts and the application of an abundance cut-off of 0.001%, 8,991,033 sequences and 10,099 OTUs remained. To compare samples without statistical bias, data were rarefied to 14,092 reads per sample, which reflected the lowest obtained read number. Rarefaction was performed to account for variations in library sizes between samples of different compartments. Rarefaction analysis indicated a sufficient sampling depth for further investigation at 97% sequence similarity (Figure S1).

3.3. Characterization of Bacterial Diversity

Analysis of α-diversity (diversity in terms of OTU numbers present in a single sample), measured as the Shannon index and chao1 richness (Figure 1), indicated a significant impact of LUI mainly on bacterial diversity in bulk soil in June, with increased values at sites with high LUI compared to sites with low LUI. For other timepoints no significant influence of LUI on bacterial diversity was detected in bulk soil. For the other compartments (rhizosphere and root interior) no significant influence of LUI on bacterial diversity was measured for any of the sampling timepoints. However, as expected, overall α-diversity was higher in the rhizosphere and bulk soil compared to the root interior.

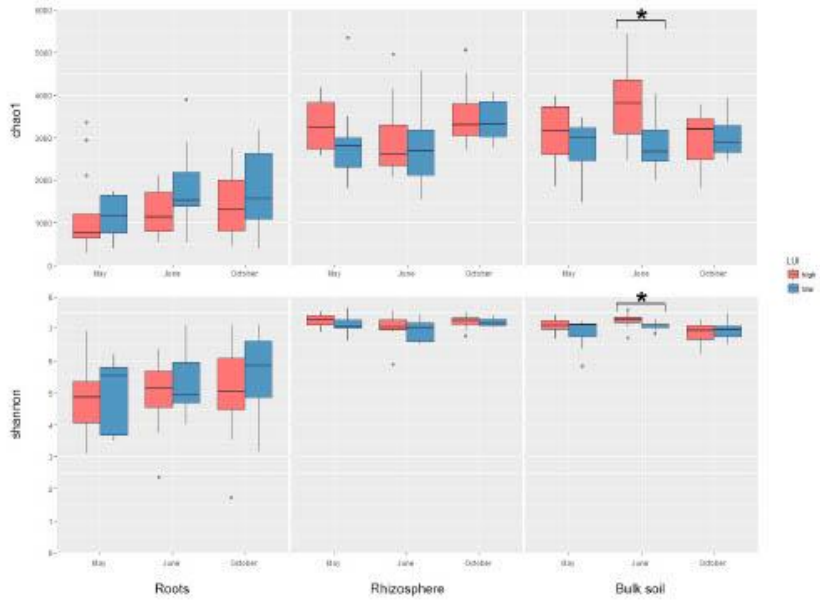

Figure 1. α-diversity measures chao1 species richness and Shannon index of all compartments under low and high land use intensity, respectively. The distribution is shown at 97% sequence similarity. The boxplot indicates the first and third quartile. The median is indicated as a horizontal line and whiskers indicate minimum or maximum, respectively. Significant differences among land use intensities are indicated with an asterisk, circles reflect outlier values (*t*-test, $p < 0.05$).

Beta-diversity was also impacted by LUI to a large extent, mainly in June, when effects of LUI on all compartments were observed. In addition, significant differences between different LUI levels were also observed in the rhizosphere and bulk soil for the other two sampling periods (Table S3). Weighted UniFrac distances indicated a significant impact of season for all analyzed compartments (Table S4).

Most abundant phyla in all compartments (endosphere, rhizosphere and bulk soil) were Proteobacteria, Bacteroidetes and Actinobacteria, followed by Firmicutes and Acidobacteria (Table S5). At the genus level (Table S6), *Pseudomonas* (23%) was dominating the root endosphere followed by *Janthinobacterium* (4%), *Rhizobium* (3%), *Burkholderia* (3%) and genera belonging to the family of Enterobacteriaceae (4%). In contrast, in the rhizosphere as well as in bulk soil, most abundant taxa were assigned to the families Sinobacteraceae (9/6%) and Chitinophagaceae (8/12%) followed by the genus *Rhodoplanes* (4/6%).

3.4. Definition of an Endophytic Core Microbiome for *Dactylis glomerata* L.

From the 88 OTUs detected in roots under high LUI on the 97% homology level, 76 were affected by the sampling timepoint. At all sampling timepoints, 12 OTUs were detected and formed a plant-associated bacterial core community (high LUI, May, June and October; Figure 2).

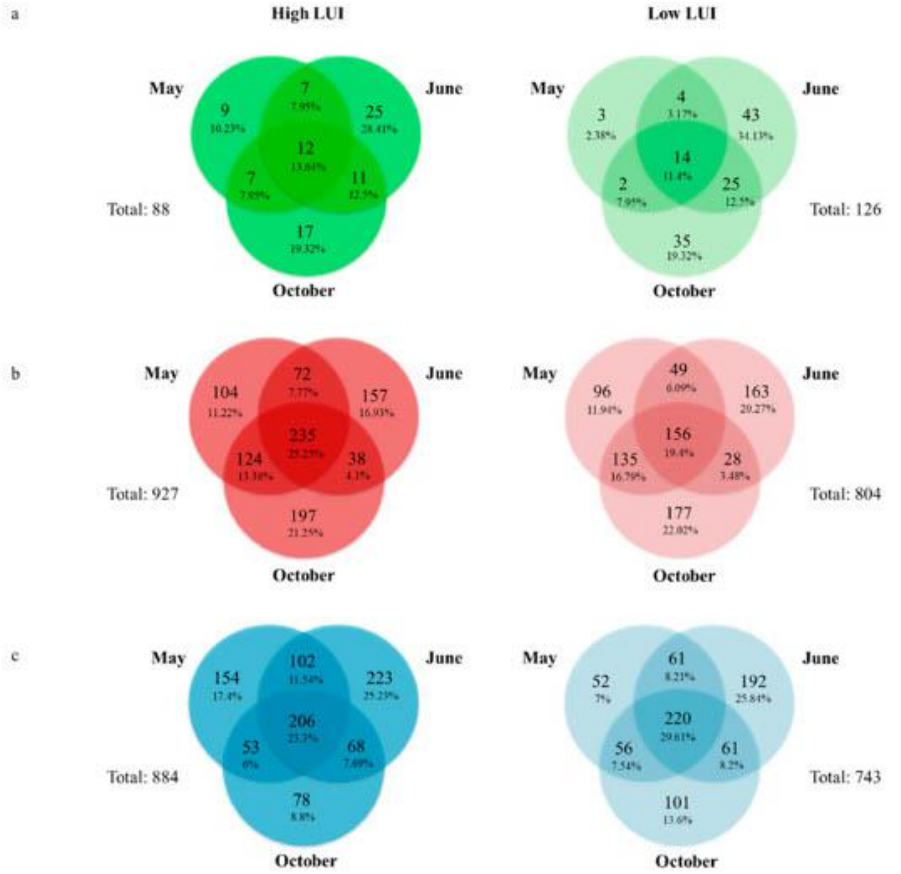

Figure 2. Shared operational taxonomic units (OTUs) between the different sampling seasons May, June and October for both land use intensities (LUIs). Green depicts root samples (a), red depicts rhizosphere samples (b) and blue depicts bulk soil samples (c). The percentages are based on the total amount of OTUs that contribute to the diagram.

Five of these OTUs were classified as *Pseudomonas* without a clear affiliation to a particular species (OTU 398604, 4455861, 3314521, 9448 and 1087); one OTU (633252) could be further classified as *P. veronii*. The other OTUs of the core microbiome could be assigned to *Rhizobium* (OTU 1104627, 220539), *Bradyrhizobium* (OTU 4377104, 1105814), *Agrobacterium* (OTU 969805) and *Labrys* (OTU 218772; see Table S7).

Overall, more OTUs could be detected in roots under low LUI compared to high LUI (126 OTUs compared to 88 on the level of 97% homology). However, the plant-associated core bacterial communities including all sampling timepoints (low LUI, May, June and October) were comparable (14 OTUs compared to 12; Figure 2). The classification revealed close similarities between the plant-associated core bacterial communities of high and low LUI. Like for high LUI, most OTUs in the core (8) could be identified as *Pseudomonas*. The OTU assigned to *P. veronii* for both land use intensities were identical (OTU 633252). Other identical OTUs found were OTUs 3,314,521, 9448 and 1087, classified also as *Pseudomonas* and OTU 4,377,104 and 1,105,814, assigned to *Bradyrhizobium*. OTUs that

were unique in the core bacterial composition of plants from low LUI sites, but phylogenetically comparable to OTUs found in the roots of plants from high LUI sites were OTUs 12,056, 6025 and 14298, which were further classified to *P. umsongensis*. In addition to those, we found one OTU (646,549), classified as *Pseudomonas* and three OTUs classified as *Rhizobium* (2156, 1433, 1,104,627), which were phylogenetically similar to those detected in roots of plants from sites with high LUI.

The only OTU that was solely found as part of the root core microbiome of plants at sites with low LUIs was assigned to *Caulobacter* (OTU 7929). In contrast, the OTUs that were only present in the endophytic core microbiome of plants under high LUI were assigned to *Agrobacterium* (OTU 969805) and *Labrys* (OTU 218772; see Table S8).

3.5. Definition of a Core Microbiome for the Rhizosphere of Dactylis glomerata L.

Due to the higher diversity in the rhizosphere compared to the root interior, the absolute numbers of OTUs, which contributed to the bacterial core microbiome of the rhizosphere, were higher. However, surprisingly also relative numbers were increased (Figures 2b and 3b). While in the root interior the bacterial core composition was only formed by 11–13% of the detected OTUs, more than 19% of the detected OTUs were part of the plant-associated core bacterial communities in the rhizosphere (Figure 2a,b). Interestingly, the numbers, as well as the proportion of OTUs that contributed to the core that was shared amongst all sampling seasons in the rhizosphere was lower under low LUI (156/19.4%) compared to high LUI (235/25.3%). In contrast to the root core bacterial community composition (shared throughout all sampling seasons under high vs. low LUI), the amount of uniquely found OTUs was higher under high LUI compared to low LUI (Figure 3b).

While the core plant-associated bacteria (found across all sampling seasons) in roots were dominated by OTUs, which could be assigned to *Pseudomonas* under both LUIs, OTUs linked to this genus were less abundant in the core bacterial community (found across all sampling seasons) of the plant rhizosphere independent of LUI. Under both LUIs, the majority of core OTUs was assigned to the genera *Rhodoplanes*, *Methylibium*, *Kaistobacter* and *Bradyrhizobium*, and to the families Sinobacteraceae and Chitionophagaceae.

We found a total number of 927 OTUs under high LUI in the rhizosphere, where 692 OTUs were influenced by a sampling timepoint on a 97% sequence similarity level (not found across every season). 235 OTUs were detected independent of the sampling timepoint, reflecting the high LUI rhizosphere core (found across all seasons). The majority of OTUs found in the core was assigned to the genera *Rhodoplanes* (16 OTUs), *Methylibium* (15 OTUs), *Kaistobacter* (11 OTUs) and *Bradyrhizobium* (8 OTUs). Furthermore, a high number of not further assigned Sinobacteraceae (20 OTUs) and Chitinophagaceae (12 OTUs) was detected (Table S9).

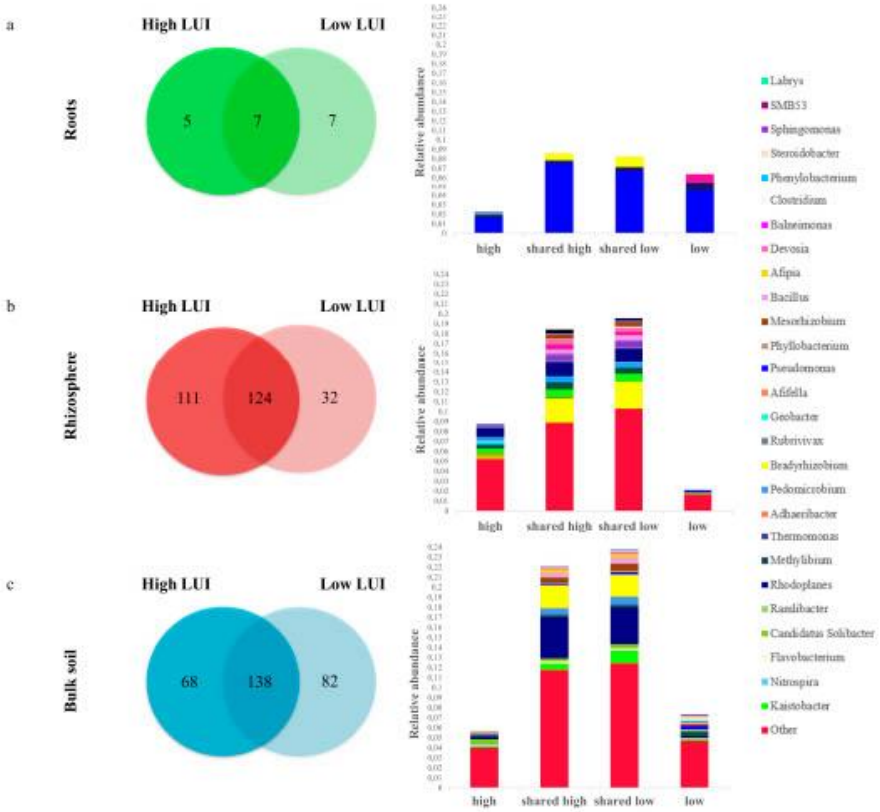

Figure 3. Comparison of plant-associated core bacterial communities. The Venn diagrams show the amount of OTUs that contributed to the core bacterial communities that were shared amongst all seasons only under high and low LUI as well as the core OTUs found under both LUIs and all seasons for roots (green, (a)), rhizosphere (red, (b)) and bulk soil (blue, (c)). The stackplots show the comparison of the total relative abundance of the OTUs that contribute to the core bacterial communities in Figure 3a. "High" refers to the abundance of OTUs found under high LUI only (shared amongst all seasons, but only under high LUI), "shared high" refers to the abundance under high LUI of those OTUs that were found under both LUIs (shared amongst all seasons under high as well as low LUI), "shared low" refers to the abundance under low LUI of those OTUs that were found under both LUIs (shared amongst all seasons under high as well as low LUI), abundance of OTUs under low LUI only (shared amongst all seasons, but only under low LUI), colored by genus. OTUs in "Other" could not be classified to the genus level.

Under low LUI, we found a total number of 804 OTUs, with 648 OTUs being influenced by the sampling date on the 97% sequence similarity level (not found acrossevery season). The plant-associated core bacterial community composition was formed by 156 OTUs (found across all seasons). As for the root core plant-associated bacteria, the largest part of the rhizosphere core (low LUI) was comparable to the core found under high LUI regarding the genera detected. In total, we found 124 identical OTUs in both high and low LUI core bacterial community composition. The genera that were found in highest amounts were the same as under high LUI: *Rhodoplanes* (11 out of 12 OTUs identical to high LUI core), *Kaistobacter* (7 out of 8 OTUs identical), *Methylibium* (7 identical OTUs) and

Bradyrhizobium (7 identical OTUs). Additionally, not further classified families that were found in high numbers were the same: Sinobacteraceae (11 identical OTUs) and Chitinophagaceae (5 out of 8 OTUs identical). Thirty-two OTUs appeared to be unique in the rhizosphere core of low LUI. Among those were the genera *Pseudomonas* (OTUs 646,549, 398,604), *Candidatus Solibacter* (OTU 817,874), *Flavobacterium* (OTU 6614), *Kaistobacter* (OTU 331,282), *Niabella* (7994), *Novosphingobium* (OTU 941,803), *Pedobacter* (OTU 976,441) and *Rhodoplanes* (OTU 2,025,156). Moreover, six unique OTUs could be classified to a particular species (on the 97% homology level; Table S10, Table S11 and Table S12): *Asticcacaulis biprosthecium* (OTU 1,105,085), *Bosea genosp.* OTUs (567,840, 829,415), *Variovorax paradoxus* (OTUs 123, 2575) and *Sphingomonas wittichii* (OTU 3395). Additionally, we found three taxa that were only present in the low LUI plant-associated core bacterial community composition: *Novosphingobium* (OTU 94,180), *Niabella* (OTU 7994) and *Pedobacter* (OTU 976,441).

OTUs unique to the high LUI rhizosphere core (111) appeared to be more diverse compared to low LUI (Figure 3b). Within the 111 unique OTUs in the core of high LUI, 39 OTUs could be assigned to genus level without further classification to the species. Among those we found *Methylibium* (8 OTUs), *Rhodoplanes* (5 OTUs), *Kaistobacter* (4 OTUs) and *Candidatus Solibacter* (3 OTUs). Furthermore, among the unique OTUs under high LUI, we found OTUs that could be assigned to *Rhizobium* (103,410), *Mycoplana* (998,905), *Labrys* (543,156), *Asticcacaulis* (1,105,085) *Adhaeribacter* (1,069,076), *Paucibacter* (593,163) *Microlunatus* (249,330, 4209) and *Bosea* (567,840, 829,415).

3.6. Definition of a Core Microbiome for the Bulk Soil of Dactylis glomerata L.

The bulk soil associated bacterial core communities were comparable to the rhizosphere associated core bacterial communities with regard to the composition as well as to the total amount of shared OTUs found amongst all sampling seasons. Under high LUI, we found in total 884 OTUs, 678 of which were influenced by the sampling timepoint (not found across all seasons). We found 206 OTUs to be shared across all sampling seasons under high LUI on the 97% sequence similarity level. The majority of OTUs assigned to the genus level was also comparable to the core bacterial community composition of the rhizosphere. OTUs in the shared core of the bulk soil (found across all seasons) were assigned to *Rhodoplanes* (23 OTUs), *Kaistobacter* (10 OTUs), *Candidatus Solibacter* (8 OTUs), *Bradyrhizobium* (8 OTUs), *Methylibium* (8 OTUs) and *Pedomicrobium* (8 OTUs). High numbers of not further classified families were *Sinobacteraceae* (22 OTUs) and *Chitinophagaceae* (16 OTUs) like in the rhizosphere. Genera found to be unique in the core of high LUI were *Geobacter* (701,911), *Ramlibacter* (4928), Afifella (10,984), *Nitrospira* (173,004), *Adhaeribacter* (1,069,076) and *Thermomonas* (805,685). Only the latter two OTUs were also found in the high LUI core bacterial community composition of the rhizosphere.

Under low LUI, a total of 743 OTUs was detected across all seasons in the bulk soil. Among those, 220 could be found independent of the sampling timepoint (shared across all sampling seasons). Most OTUs could be classified to *Bacillus* (with 3 out of 5 OTUs being identical to high LUI OTUs), *Bradyrhizobium* (8 out of 9 identical OTUs), *Kaistobacter* (6 out of 8 identical OTUs), *Methylibium* (4 out of 9 identical OTUs), *Pedomicrobium* (7 out of 8 identical OTUs), *Rhodoplanes* (19 out of 22 identical OTUs), *Steroidobacter* (1 out of 7 identical OTUs), *Sinobacteraceae* (16 out of 25 identical OTUs) and *Chitinophagaceae* (8 out of 11 identical OTUs). In addition to this, two genera were found solely present in the low LUI core: SMB53 (555,945) and Labrys (543,156). OTU 543,156, however, was found in the core bacterial community composition of low LUI rhizosphere, too.

4. Discussion

4.1. The Role of LUI for Bacterial Diversity at the Plant–Soil Interface at Different Stages of Plant Development

In this study, we compared the putative core bacterial communities of the agricultural important grass *Dactylis glomerata* L. under the influence of different LUIs. Plants were sampled at three different dates from sites exposed to high and low LUI, respectively, to characterize the bacterial community composition in the endosphere and rhizosphere as well as in the bulk soil.

The analysis of α-diversity showed an increase of microbial diversity under high LUI within all plant stages only in the bulk soil. This might be explained by a more transient and patchy bacterial colonization under higher LUI [33]. In contrast, under low land use intensity, bacterial communities are differing strongly between the measured timepoints during the season, indicating a clear fluctuation of bacterial communities over the season, which is not the case under high land use intensity. Lower α-diversity as a result of low LUI, which we attribute to less disturbance, results in a more stable and consistent composition of bacterial communities [34]. However, we could neither find a significant impact of LUI on the bacterial α-diversity in the rhizosphere, nor in the root endosphere. This is in accordance with several studies showing that plant species have a stronger effect on plant-associated microbial communities than soil parameters [35,36].

Analysis of β-diversity within the root endosphere revealed a significant impact of land use intensity in June, indicating a difference in the presence and/or absence of certain OTUs. This might be due to seed production in June, which generates high metabolic expenses for the plant, which are caused by the synthesis of storage products (including proteins, starch and lipids), the uptake of mineral nutrients, as well as nutrient translocation from the site of synthesis to seed assimilation [37]. Thus, mainly at sites with low land use intensity and reduced amounts of plant available nitrogen, it is essential for optimal performance of the plant to recruit bacteria, which are capable of forming plant available nitrogen either by mineralization of dead biomass or by nitrogen fixation, which might explain differences in β-diversity in June between sites with different land use intensities.

As expected, a significant difference between LUIs was also observed in the rhizosphere and bulk soil at every sampling date. In addition, we also found a significant difference in the presence and/or absence of certain OTUs (unweighted Unifrac) between different sampling dates, within high and low LUI, respectively, in all compartments (Table S8). Thus, the results suggest a root-associated bacterial community composition that is selected by the plant throughout the season, which is in accordance with numerous studies [2,38,39] and additional effects driven by LUI, which differ in their degree and direction towards shifts in the abundance of single OTUs dependent on the sampling date. As the LUI index we used in our study takes into account various parameters, which are affected by land use intensity, including fertilization and subsequent changes of nitrogen pools in soils, the observed LUI-mediated effects might be coupled to altered soil parameters, which in turn affected the bacterial community composition.

4.2. The LUI Independent Bacterial Core Microbiome of *D. glomerate*

The main focus of this study was to identify a putative plant-associated core bacterial community of *D. glomerata* in different plant compartments, because bacteria that are consistently found across samples subjected to different conditions likely provide critical ecological functions. Indeed, we found plant-associated bacterial communities that were shared across all sampling seasons under both LUIs (Figure 2). *Pseudomonas veronii* was found in the root core of both LUIs, emphasizing its importance for *D. glomerata*. Previous studies have demonstrated that root-associated *P. veronii* exhibits high biocontrol potential by increasing the bioavailability of phosphate and ammonia in the soil [40] and by synthesizing indole-3-acetic acid (IAA), which is of major importance, since it stimulates cell elongation and cell division of the plant [41]. Furthermore, a high nematodical activity was observed within this species [42]. It was shown that the abundance of plant-parasitic nematodes is significantly increased in *D. glomerata* compared to other grasses and legumes [43]. This might be critical for *D. glomerata* since it can affect the competitive ability of the plant significantly. Moreover, other *Pseudomonas* spp. were reported to possess a wide variety of plant growth promoting traits and beneficial properties. These include the production of various phytohormones like IAA, cytokinins or gibberillins, as well as nitrogen fixation and production of antimicrobial compounds [44]. Additionally, *Rhizobium* and *Bradyrhizobium* were found in the plant-associated core bacterial composition independent of LUI. Both genera are known for their nitrogen-fixing abilities within nodules of leguminous plants [45,46] and for the production of phytohormones [47,48]. Moreover, genes for nitrogen fixation of the endophytic

Rhizobium spp. were found in high abundances within tissues of the perennial grass sugarcane, indicating their particular importance for plant-associated nitrogen fixation in perennial grasses [49]. Due to its high abundance in roots, it is presumably a key player for plant health.

In general, more OTUs were found in total and relative numbers in the core bacterial communities (shared across all seasons, high vs. low LUI) of the rhizosphere compared to roots, which seems reasonable, as there is no obstacle to overcome like passing the cell wall of the plant. Interestingly, the most prominent genus found in root core bacterial communities (*Pseudomonas*) was not as ubiquitously found in the rhizosphere core under both LUIs. This might be due to higher competition and the strong adaptation of *Pseudomonas* spp. to the specific conditions present in the root interior. The majority of genera found in the rhizospheric cores (high and low LUI) were *Rhodoplanes*, *Methylibium*, *Kaistobacter* and *Bradyrhizobium*, which were frequently isolated from other rhizosphere environments [50–53]. Among those, *Bradyrhizobium* is the only one that was also found in the root core bacterial communities under both LUIs, highlighting its importance for the plant. While representatives of *Rhodoplanes* have been characterized as a facultative photo-organothroph and potentially nitrate-fixing bacteria [54,55], *Methylibium* was described as a facultative methylotroph that actively utilizes root exudates [51]. Furthermore, species within the latter are involved in the degradation of aromatic hydrocarbon and methyl tert-butyl ether [50]. Members of *Kaistobacter* have also been reported to be involved in the degradation of aromatic compounds and suggested to suppress bacterial wilt disease [56].

4.3. Microbial Variation of Core OTUs as Influenced by LUI

In addition to the LUI-independent plant-associated core bacterial community composition (shared throughout all seasons and both LUIs), we also found taxa that were only present in the core under high or low LUI (shared throughout all seasons, but only on high or low LUI). In roots, the OTU assigned to *Caulobacter* was present exclusively in all samples from low LUI sites, constituting part of the core bacterial communities in roots. Microbes within this genus were reported to produce IAA and solubilize inorganic phosphate [57]. A study on bacterial communities in different grapevine cultivars showed higher abundance of *Caulobacter* under organic production versus integrated pest management [58], which would be in line with our findings of *Caulobacter* under low management intensity. In turn, a single OTU assigned to *Labrys* was found exclusively in the core of sites subjected to high LUI (shared throughout all seasons on high LUI). Species within this genus are frequently isolated from various soil and sediment samples but were also found in the rhizosphere of Korean ginseng [59–61], and as an endophyte in *Clerodendrum colebrookianum* [62]. Though no strain within this genus has been described in the context of plant growth-promoting traits, numerous species of *Labrys* isolated from sediment and soil were shown to possess the ability to reduce nitrate, assimilate various amino acids and sugars, have catalase activity etc. [59,60], which might also play an important role in supporting the plant under a high amount of available nitrate. *Agrobacterium* (e.g., *Agrobacterium tumefaciens*) is widely known for its ability to transmit plasmid T-DNA into plant cells [3]. Furthermore, several *Agrobacterium* species carry pathogenic capacity on Ti (tumorigenic) or Ri (rhizogenic) plasmids, which can cause the induction of tumor-like growth and reduce seed production, e.g., crown-gall or hairy root disease [63]. On the other hand, various nonpathogenic *Agrobacterium* spp. have been found lacking those plasmids [64]. Indeed, numerous *Agrobacterium* spp. appeared to contribute to plant growth by phosphate solubilization, nitrogen fixation and siderophore production [65].

We found several differences in the composition of core bacterial communities in the rhizosphere between high and low LUI, e.g., species within *Variovorax* were only found in the core of low LUI (shared throughout all seasons). *Variovorax paradoxus* is frequently described as the plant-growth promoting genus, including traits like the reduction of plant stress, increasing nutrient availability and inhibiting growth of pathogens by degrading N-acyl homoserine-lactones. The latter constitute mechanisms related to their catabolic capacities [65–67], which are likely critical to satisfy the metabolic expenses of the plant as they are found in the core microbiome of low LUI. Furthermore, *Sphingomonas wittichii* and *Bosea genosp* were only found in the low LUI core (shared throughout all seasons). Numerous studies

showed their plant-growth promoting abilities due to their production of phytohormones [68,69]. Moreover, other species found only in the low LUI core within the genera *Sphingomonas* and *Bosea* were shown to be diazotrophic, solubilize inorganic phosphorus and are involved in biocontrol [70]. *Asticcacaulis biprosthecium* has recently been found in the rhizosphere of maize, however, their plant-growth promoting abilities have not been further described [71].

Genera exclusively found under high LUI, but shared throughout all sampling seasons were *Rhizobium*, *Mycoplana*, *Labrys*, *Adhaeribacter*, *Paucibacter* and *Microlunatus*, all of which have been described to harbor plant-growth promoting functions and thus enhancing plant performance [72–77].

OTUs found in the rhizosphere and bulk samples differed as expected, but all taxa that were detected in the bulk soil core bacterial communities were also found in the rhizosphere core under both LUIs. Differences in the core of bulk soil and the rhizosphere may be due to the selective attraction of taxa by the plant. Interestingly, LUI influenced the proportion of OTUs that contributed to the uniquely found OTUs (shared among all sampling seasons, but only on high or low LUI) and on the number of taxa in the rhizosphere (total OTUs found throughout all seasons), as OTUs were assigned to more different taxa under high LUI. Though the prevalent taxa specific for one plant stage were comparable in the rhizosphere, the amount and proportion of OTUs contributing to the core were lower under low LUI (156/19.4%) compared to high LUI (235/25.35%). Furthermore, the higher amount of OTUs that were present in the low LUI associated core bacterial communities compared to high LUI indicates a higher variability of bacteria colonizing the rhizosphere. In a low nutrient environment, specific recruitment of microorganisms is crucial to the plant to enhance plant fitness and growth [78]. Thus, a lower amount of similar genera may be found, indicating a more selective attraction of soil bacterial communities under low LUI by plant exudation throughout the season. Furthermore, the observation that seven OTUs were present in root samples and 124 in rhizosphere samples independent of LUI and sampling timepoints imply that these taxa are highly persistent and ubiquitous in agricultural soil.

5. Conclusions

During the last years, several studies investigated the influence of land management on plant-associated bacteria, thereby focusing on single plant development stages or compartments. As frequently occurring plant-associated bacterial assemblages are presumably critical for plant fitness [18], the identification of a plant-associated core bacterial community composition might be the first step of defining a "healthy" bacterial community to unravel the ecology of plant-associated bacterial consortia and predict community responses to environmental changes. Up to date, this is the first study defining the composition of a stable plant-associated core bacterial community composition in the rhizosphere and endosphere as well as its dependency on LUI for a plant species of agricultural importance. We found the genera *Pseudomonas*, *Rhizobium* and *Bradyrhizobium* to be part of the consistent root core plant-associated bacteria of *D. glomerata* independent of LUI and stable along the season. The majority of genera identified as the core bacterial communities of the rhizosphere compartment belonged to *Rhodoplanes*, *Methylibium*, *Kaistobacter* and *Bradyrhizobium*. Their persistent occurrence independent of LUI or the growth stage as well as their plant-growth promoting traits supporting plant health could be a first insight into the composition of a life-supporting essential part of the plant microbiome. Nevertheless, we could not infer from the measured co-occurrence of plant-associated bacterial communities across different LUI how the detected species react and interact with their host plants, although many of the identified taxa have been associated with plant growth promoting traits in previous studies [79]. In this context, also the co-occurrence of fungi and bacteria especially in the plants' rhizosphere compartment should be assessed to account for the presence and contribution of fungi in plant-associated microbiomes.

A higher compositional variation throughout different sampling dates was observed under low LUI compared to high LUI, suggesting a stronger adaption of plant-associated bacteria under low LUI. Taking the minimum cut-off of 0.001% (i.e., required number of reads for one OTU to be kept was 103) after cut-off into account, these results were significant.

Taking the functions associated to the detected bacterial species into account, we could speculate that the major functionality of the plant-associated core bacterial community might be linked in particular to the plant growth and stress response. However, to obtain deeper insights into the functionality of a "healthy" core bacterial microbiome and its resilience to disturbances, it is of importance to further analyze the functional traits of the identified microbial communities to reveal their functional potential. This will gain deeper knowledge on core plant-associated bacteria of a healthy versus a diseased state of the plant and the predictability of the fitness state of the plant to improve plant performance in agricultural production systems. Though our study revealed the existence of shared plant-associated bacterial taxa under different land use intensities, these findings were specific for *D. glomerata* in the investigated grassland soils. Thus, it would be tempting to assess also bacterial communities associated with other *D. glomerata* cultivars or to study broader biogeographical patterns of the root associated microbiome of *D. glomerata* or other grass species of Poaceae.

Supplementary Materials: The following materials are available online at http://www.mdpi.com/1424-2818/12/10/392/s1, Figure S1: Rarefaction curves, Table S1: Summary of sampling plot parameters, Table S2: Summary of edaphic parameters, Table S3: The impact of LUI on the β-diversity within one sampling season in all compartments (between sample diversity), Table S4: The impact of sampling season on the β-diversity within one LUI in all compartments (between sample diversity), Table S5: Relative abundance of assigned phyla, Table S6: Relative abundance of assigned genera, Table S7: Classification of core OTUs in roots and high LUI. Shared core OTUs (May, June and October) high LUI: 12, Table S8: Classification of core OTUs in roots and low LUI. Shared core OTUs (May, June and October) low LUI: 14, Table S9: Classification of shared core OTUs that were found in 95% in high LUI samples across all sampling seasons (rhizosphere), Table S10: Classification of shared core OTUs that were found in 95% in low LUI samples across all sampling seasons (rhizosphere), Table S11: Classification of shared core OTUs that were found in 95% in high LUI samples across all sampling seasons (bulk soil), Table S12: Classification of shared core OTUs that were found in 95% in high LUI samples across all sampling seasons (bulk soil).

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Supplemental Information Manuscript 2

Supplemental Material:

Figure S1: Rarefaction curves

Table S1: Summary of sampling plot parameters

Table S2: Summary of edaphic parameters

Table S3: The impact of LUI on the β-diversity within one sampling season in all compartments (between sample diversity)

Table S4: The impact of sampling season on the β-diversity within one LUI in all compartments (between sample diversity)

Table S5: Relative abundance of assigned Phyla

Table S6: Relative abundance of assigned Genera

Table S7: Classification of core OTUs in roots and high LUI. Shared Core OTUs (May, June, October) high LUI: 12

Table S8: Classification of core OTUs in roots and low LUI. Shared Core OTUs (May, June, October) low LUI: 14

Table S9: Classification of shared core OTUs that were found in 95% in high LUI samples across all sampling seasons (rhizosphere)

Table S10: Classification of shared core OTUs that were found in 95% in low LUI samples across all sampling seasons (rhizosphere)

Table S11: Classification of shared core OTUs that were found in 95% in high LUI samples across all sampling seasons (bulk soil)

Table S12: Classification of shared core OTUs that were found in 95% in high LUI samples across all sampling seasons (bulk soil)

Figure S1

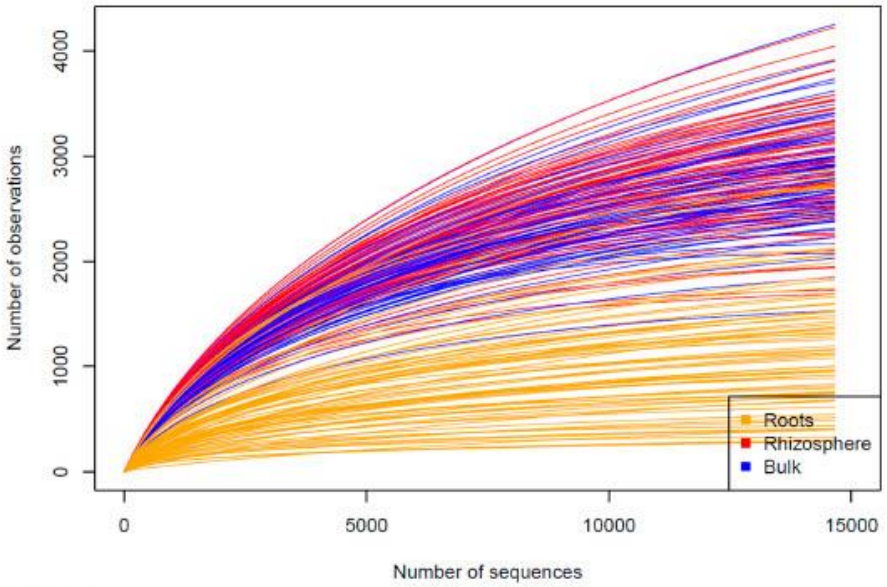

Table S1

Plot ID	Management	Treatment	Index (2006-2014)
AEG6	mown pasture	fertilized	2.25
AEG19	mown pasture	fertilized	2.28
AEG20	pasture	fertilized	1.55
AEG21	meadow	fertilized	3.62
AEG7	meadow	non-fertilized	0.56
AEG28	pasture	non-fertilized	1.06
AEG33	pasture	non-fertilized	1.32
AEG34	pasture	non-fertilized	1.31

Table S2

Timepoint	Plot ID	WEOC µg g-1 dw	WEON µg g-1 dw	Nitrate µg N g-1 dw	Ammonium µg N g-1 dw	C/N ratio plants	Water (%)	Total C g/kg soil	Total N g/kg soil
May	AEG6	36.99±2.28	26.79±7.57	21.02±7.17	0.13±0.03	7.56±0.6	36	88,21	8,71
	AEG19	41.45±5.40	32.91±8.04	24.73±6.23	0.18±0.03	7.73±0.42	41	82,64	8,09
	AEG20	33.05±2.59	14.08±1.48	8.51±1.62	0.18±0.05	13.06±2.31	41	86,19	9,17
	AEG21	33.49±3.12	13.41±5.24	11.84±2.82	0.71±0.2	10.86±0.94	31	70,77	7,06
	AEG7	34.05±3.14	6.24±2	2.09±0.8	0.31±0.05	12.96±2.34	36	88,69	4,77
	AEG28	37.79±2.75	5.55±1.8	2.28±1.38	0.44±0.09	21.41±3.82	34	82,64	8,09
	AEG33	31.44±9.08	15.02±5.09	10.54±2.68	0.83±0.44	17.81±2.2	35	86,19	9,17
	AEG34	32.07±4.28	8.09±1.59	3.17±1.7	0.42±0.27	16.11±4.32	33	70,77	7,06
June	AEG6	25.84±5.55	40.65±8.23	42.29±4.53	0.1±0.04	19.73±2.78	37	88,21	8,71
	AEG19	30.25±7.8	41.87±9.41	45.57±9.13	0.11±0.03	12.25±0.19	34	82,64	8,09
	AEG20	24.75±4.76	48.42±11.92	51.04±7.26	0.11±0.02	13.04±1.41	44	86,19	9,17
	AEG21	25.56±4.5	41.57±12.2	45.43±11.04	0.13±0.06	14.51±1.39	36	70,77	7,06
	AEG7	27.54±3.5	25.61±5.79	17.03±4.87	5.76±1.57	16.65±0.21	40	88,69	4,77
	AEG28	25.08±6.02	14.46±3.8	12.11±2.93	2.28±0.49	25.78±1.85	34	82,64	8,09
	AEG33	25.48±4.65	8.67±1.56	4.55±2.48	2.81±0.91	26.46±1.27	31	86,19	9,17
	AEG34	24.05±4.71	21.25±4.59	24.77±5.19	0.79±0.38	28.33±0.28	30	70,77	7,06
October	AEG6	58.64±3.84	13.04±2.67	6.12±4.78	1.7±0.28	11.57±2.01	26	88,21	8,71
	AEG19	86.1019.19	39.85±12.36	8.39±3.21	2.5±0.82	9.32±1.35	31	82,64	8,09
	AEG20	40.86±4.77	59.6±4.53	19.6±6.67	0.039±0.031	12.17±2.56	38	86,19	9,17
	AEG21	81.49±7.89	25.94±5.94	14.19±5.66	1.06±0.67	12.29±0.86	28	70,77	7,06
	AEG7	61.47±21.35	10.27±3.49	2.2±1.4	5.83±2.24	21.4±5.78	26	88,69	4,77
	AEG28	37.23±6.99	25.12±3.57	5.42±3.06	3.18±1.18	18.68±0.3	24	82,64	8,09
	AEG33	85.92±8.08	9.48±1.17	0.66±0.34	1.97±0.5	16.35±3.7	29	86,19	9,17
	AEG34	55.35±6.07	32.21±6.69	15.37±9.15	0.72±0.45	20.34±4.04	25	70,77	7,06

Table S3

Compartment	Sampling season	Unifrac Distance	R2	p-value	Significance level
Roots	May	weighted	0.04643	0.311	
		unweighted	0.0498	0.154	
	June	weighted	0.04626	0.349	
		unweighted	**0.06919**	**0.027**	*
	October	weighted	0.05816	0.192	
		unweighted	0.05056	0.176	
Rhizosphere	May	weighted	0.04955	0.317	
		unweighted	**0.06709**	**0.031**	*
	June	weighted	0.06403	0.155	
		unweighted	**0.08079**	**0.005**	**
	October	*weighted*	*0.09016*	*0.085*	.
		unweighted	**0.08579**	**0.007**	**
Bulk	May	**weighted**	**0.12267**	**0.037**	*
		unweighted	**0.11207**	**0.001**	***
	June	**weighted**	**0.08407**	**0.05**	*
		unweighted	**0.13933**	**0.002**	**
	October	weighted	0.0569	0.246	
		unweighted	**0.08958**	**0.016**	*

Table S4

Compartment	LUI	Unifrac Distance	R2	p-value	Significance level
Roots	high	*weighted*	*0.0928*	*0.068*	.
		unweighted	**0.09197**	**0.011**	*
	low	weighted	0.08401	0.154	
		unweighted	**0.08175**	**0.027**	*
Rhizosphere	high	**weighted**	**0.13248**	**0.009**	**
		unweighted	**0.13786**	**0.001**	***
	low	**weighted**	**0.15565**	**0.001**	***
		unweighted	**0.16921**	**0.001**	***
Bulk soil	high	**weighted**	**0.16153**	**0.015**	**
		unweighted	**0.14025**	**0.001**	***
	low	*weighted*	*0.10641*	*0.066*	.
		unweighted	**0.09614**	**0.004**	**

Table S5

Phylum	Roots RA (%)	Rhizosphere RA (%)	Bulk RA (%)
Acidobacteria	0.011	0.033	0.046
Actinobacteria	0.029	0.069	0.098
Bacteroidetes	0.058	0.171	0.198
Chlamydiae	<0.001	0.000	0.001
Chlorobi	0.001	0.004	0.010
Chloroflexi	<0.001	0.001	0.002
Fibrobacteres	<0.001	0.001	<0.001
Firmicutes	0.012	0.012	0.019
Gemmatimonadetes	0.001	0.007	0.009
Nitrospirae	0.002	0.006	0.013
Planctomycetes	0.001	0.003	0.004
Proteobacteria	0.880	0.679	0.583
Spirochaetes	<0.001	<0.001	0.001
TM6	<0.001	0.002	0.004
TM7	0.002	0.011	0.011
WS3	<0.001	<0.001	0.001
Other	0.003	0.001	0.002

Table S6

Taxon	Roots RA (%)	Rhizosphere RA (%)	Bulk RA (%)
Other	0.13	0.19	0.19
Agrobacterium	0.02	0.00	0.00
Bacillus	0.00	0.01	0.01
Bosea	0.01	0.00	0.00
Bradyrhizobium	0.02	0.03	0.03
Burkholderia	0.03	0.00	0.00
Candidatus Solibacter	0.00	0.01	0.02
Caulobacter	0.01	0.01	0.00
Citrobacter	0.02	0.00	0.00
Devosia	0.00	0.01	0.00
Dokdonella	0.00	0.01	0.00
Enterobacter	0.01	0.00	0.00
Erwinia	0.01	0.00	0.00
Flavobacterium	0.00	0.01	0.01
Gluconacetobacter	0.03	0.00	0.00
Janthinobacterium	0.04	0.01	0.00
Kaistobacter	0.00	0.02	0.02
Labrys	0.01	0.00	0.00
Luteibacter	0.02	0.00	0.00
Mesorhizobium	0.01	0.01	0.01
Methylibium	0.02	0.02	0.01
Microlunatus	0.00	0.01	0.01
Nitrospira	0.00	0.00	0.01
Pedobacter	0.01	0.01	0.00
Pedomicrobium	0.00	0.01	0.01
Polaromonas	0.01	0.01	0.00
Pseudomonas	0.23	0.01	0.01
Pseudoxanthomonas	0.00	0.01	0.00
Rahnella	0.02	0.00	0.00
Ramlibacter	0.00	0.01	0.00
Rhizobium	0.03	0.00	0.00
Rhodanobacter	0.01	0.00	0.00
Rhodoplanes	0.01	0.04	0.06
Rubrivivax	0.00	0.01	0.01
Sphingomonas	0.02	0.01	0.00
Steroidobacter	0.00	0.00	0.01
Thermomonas	0.00	0.01	0.00
Variovorax	0.01	0.01	0.00
Xanthomonas	0.02	0.00	0.00
Alcaligenaceae__f	0.00	0.01	0.01
C111__f	0.00	0.01	0.01
Chitinophagaceae__f	0.02	0.08	0.12
Comamonadaceae__f	0.01	0.02	0.01
Coxiellaceae__f	0.01	0.00	0.00
Cytophagaceae__f	0.00	0.02	0.02

EB1017__f	0.00	0.01	0.01
Enterobacteriaceae__f	0.03	0.00	0.00
Gaiellaceae__f	0.00	0.01	0.02
Haliangiaceae__f	0.01	0.01	0.02
Hyphomicrobiaceae__f	0.01	0.01	0.02
Oxalobacteraceae__f	0.01	0.00	0.00
Rhizobiaceae__f	0.01	0.00	0.00
Rhodospirillaceae__f	0.01	0.02	0.02
Saprospiraceae__f	0.00	0.01	0.01
Sinobacteraceae__f	0.03	0.09	0.06
Sphingomonadaceae__f	0.02	0.02	0.00
Syntrophobacteraceae__f	0.00	0.01	0.01
Xanthomonadaceae__f	0.01	0.03	0.02
Acidimicrobiales__o	0.00	0.01	0.01
Actinomycetales__o	0.00	0.00	0.01
Ellin6067__o	0.00	0.03	0.03
MND1__o	0.00	0.01	0.01
Myxococcales__o	0.01	0.03	0.03
Rhizobiales__o	0.00	0.01	0.01
SC-I-84__o	0.00	0.03	0.03
Solibacterales__o	0.00	0.01	0.01
Solirubrobacterales__o	0.00	0.01	0.01
Sphingobacteriales__o	0.00	0.02	0.03
Betaproteobacteria__c	0.00	0.02	0.02
Gemm-1__c	0.00	0.00	0.01
SJA-28__c	0.00	0.00	0.01
TM7-1__c	0.00	0.01	0.01

Table S7

OTU ID	Phylum	Class	Order	Family	Genus	Species
633252	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*veronii*
398604	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
4455861	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
3314521	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.5.CleanUp.ReferenceOTU9448	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.6.CleanUp.ReferenceOTU1087	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
4377104	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1105814	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1104627	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	not assigned
220539	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	not assigned
969805	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	not assigned
218772	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Labrys*	not assigned

Table S8

OTU ID	Phylum	Class	Order	Family	Genus	Species
New.10.CleanUp.ReferenceOTU12056	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*umsongensis*
New.8.CleanUp.ReferenceOTU6025	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*umsongensis*
New.10.CleanUp.ReferenceOTU14298	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*umsongensis*
633252	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*veronii*
646549	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
3314521	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.5.CleanUp.ReferenceOTU9448	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.6.CleanUp.ReferenceOTU1087	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.7.CleanUp.ReferenceOTU2156	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	not assigned
New.10.CleanUp.ReferenceOTU1433	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	not assigned
1104627	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	not assigned
4377104	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1105814	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
New.6.CleanUp.ReferenceOTU7929	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	not assigned

Table S9

Rhizosphere: Shared Core OTUs (May, June, October) high LUI: 235						
OTU ID	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
1052559	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*asaccharolytica*
1105085	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Asticcacaulis*	*biprosthecium*
567840	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bosea*	*genosp.*
829415	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bosea*	*genosp.*
731707	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	*paradoxus*
New.3.CleanUp.ReferenceOTU123	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	*paradoxus*
New.3.CleanUp.ReferenceOTU2575	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	*paradoxus*
New.2.CleanUp.ReferenceOTU3395	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*wittichii*
1069076	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Adhaeribacter*	not assigned
969805	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	not assigned
850572	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	not assigned
838594	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
961922	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
580703	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
681987	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Balneimonas*	not assigned
318609	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573135	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
236922	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
98457	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573764	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
4377104	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1105814	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
580625	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
136297	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus solibacter*	not assigned
New.3.CleanUp.ReferenceOTU7152	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus solibacter*	not assigned
New.4.CleanUp.ReferenceOTU3180	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus solibacter*	not assigned

811449	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	not assigned
866365	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	not assigned
849118	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
573013	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
New.0.CleanUp.ReferenceOTU4830	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
1072673	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
941487	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	not assigned
166064	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	not assigned
989109	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
1109964	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
136151	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
343503	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
573258	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.0.CleanUp.ReferenceOTU6126	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.0.CleanUp.ReferenceOTU8268	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.1.CleanUp.ReferenceOTU2902	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.2.CleanUp.ReferenceOTU4934	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.3.CleanUp.ReferenceOTU6571	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.4.CleanUp.ReferenceOTU9070	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
543156	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Labrys*	not assigned
620684	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
547097	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
571263	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
4396454	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
689696	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
583766	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
672144	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU2979	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU3919	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU6392	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned

New.0.CleanUp.ReferenceOTU7272	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU7828	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.1.CleanUp.ReferenceOTU2316	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.3.CleanUp.ReferenceOTU3379	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.3.CleanUp.ReferenceOTU3915	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.3.CleanUp.ReferenceOTU4401	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.4.CleanUp.ReferenceOTU5167	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.4.CleanUp.ReferenceOTU11737	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
249330	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	*Microlunatus*	not assigned
New.0.CleanUp.ReferenceOTU4209	Actinobacteria	Actinobacteria	Actinomycetales	Propionibacteriaceae	*Microlunatus*	not assigned
998905	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Mycoplana*	not assigned
593163	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Paucibacter*	not assigned
207866	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
210344	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.2.CleanUp.ReferenceOTU211	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
279358	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
New.0.CleanUp.ReferenceOTU8673	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
New.1.CleanUp.ReferenceOTU1050	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
345333	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Phyllobacterium*	not assigned
211171	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Polaromonas*	not assigned
New.3.CleanUp.ReferenceOTU5359	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Polaromonas*	not assigned
3314521	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
252345	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Pseudoxanthomonas*	not assigned
New.0.CleanUp.ReferenceOTU4429	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ramlibacter*	not assigned
New.0.CleanUp.ReferenceOTU10000	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ramlibacter*	not assigned
New.1.CleanUp.ReferenceOTU1726	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ramlibacter*	not assigned
103410	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobium*	not assigned
222792	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
358785	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
317632	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned

545247	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
616682	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
1638797	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
210914	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.0.CleanUp.ReferenceOTU4241	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.0.CleanUp.ReferenceOTU9193	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU99	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU1979	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.2.CleanUp.ReferenceOTU3236	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU2305	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU3855	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU1289	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU1675	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU802	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.1.CleanUp.ReferenceOTU915	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.4.CleanUp.ReferenceOTU8738	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
1003206	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
New.0.CleanUp.ReferenceOTU10354	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
805685	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	not assigned
New.0.CleanUp.ReferenceOTU1960	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	not assigned
New.0.CleanUp.ReferenceOTU2939	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	not assigned
995978	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	not assigned
New.3.CleanUp.ReferenceOTU6971	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	not assigned
165771	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	not assigned	not assigned
859313	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	not assigned	not assigned
4395040	Acidobacteria	Solibacteres	Solibacterales	[Bryobacteraceae]	not assigned	not assigned
759507	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU1714	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	not assigned	not assigned
New.3.CleanUp.ReferenceOTU855	Firmicutes	Bacilli	Bacillales	Bacillaceae	not assigned	not assigned
1015820	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	not assigned	not assigned

968464	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	not assigned	not assigned
New.3.CleanUp.ReferenceOTU6138	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	not assigned	not assigned
3774028	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU9791	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	not assigned	not assigned
912263	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
363617	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
545016	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
1068155	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
693348	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
241123	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2121	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU3451	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5301	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6720	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU583	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
799231	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
589483	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU274	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2355	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5418	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5587	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU11073	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.3.CleanUp.ReferenceOTU5266	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
544711	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2877	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6745	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
854368	Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	not assigned	not assigned
1027418	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6303	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	not assigned	not assigned
962736	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	not assigned	not assigned

819885	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	not assigned	not assigned
204335	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
215375	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4459	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
4424338	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
New.2.CleanUp.ReferenceOTU2676	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
220524	Actinobacteria	Actinobacteria	Actinomycetales	Nakamurellaceae	not assigned	not assigned
539866	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	not assigned	not assigned
709657	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
564004	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
810700	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
831407	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4104	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
758928	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	not assigned	not assigned
527397	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
1059762	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
4373617	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
775149	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
1106653	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU3078	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4846	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4994	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU7347	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8554	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8889	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU19	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU1526	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU2121	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU2721	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.2.CleanUp.ReferenceOTU1387	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned

New.2.CleanUp.ReferenceOTU6790	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.3.CleanUp.ReferenceOTU3355	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.3.CleanUp.ReferenceOTU5233	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.4.CleanUp.ReferenceOTU8136	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU10009	Actinobacteria	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	not assigned	not assigned
917888	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	not assigned	not assigned
1135798	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8799	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU1501	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	not assigned	not assigned
539907	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	not assigned	not assigned
142384	Proteobacteria	Betaproteobacteria	A21b	UD5	not assigned	not assigned
945478	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned
737110	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned
New.3.CleanUp.ReferenceOTU6334	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned
New.4.CleanUp.ReferenceOTU1724	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned
410072	Actinobacteria	Acidimicrobiia	Acidimicrobiales	not assigned	not assigned	not assigned
New.2.CleanUp.ReferenceOTU5595	Firmicutes	Bacilli	Bacillales	not assigned	not assigned	not assigned
222768	Proteobacteria	Alphaproteobacteria	Ellin329	not assigned	not assigned	not assigned
399818	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
591340	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
698517	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
216418	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU9	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2144	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
New.3.CleanUp.ReferenceOTU3206	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
New.4.CleanUp.ReferenceOTU12858	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
113261	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
1021984	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
832626	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
540592	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned

New.0.CleanUp.ReferenceOTU8025	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
800292	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
583294	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU10526	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
216643	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
328181	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
818282	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU1851	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2540	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6011	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8063	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
New.1.CleanUp.ReferenceOTU2366	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
New.3.CleanUp.ReferenceOTU7464	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
807804	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned
4395045	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned
205900	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned
619918	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned
570595	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned
113674	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
223126	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
901995	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
1090634	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2451	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU9530	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
583489	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU9789	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
New.2.CleanUp.ReferenceOTU150	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
New.3.CleanUp.ReferenceOTU4583	Proteobacteria	Gammaproteobacteria	not assigned	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8883	Gemmatimonadetes	Gemm-1	not assigned	not assigned	not assigned	not assigned

Table S10

Rhizosphere: Shared Core OTUs (May, June, October) low LUI: 156						
OTU ID	**Phylum**	**Class**	**Order**	**Family**	**Genus**	**Species**
1052559	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*asaccharolytica*
731707	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*	*paradoxus*
969805	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	not assigned
850572	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Agrobacterium*	not assigned
838594	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
961922	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
580703	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
681987	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Balneimonas*	not assigned
318609	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573135	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
98457	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573764	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
4377104	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1105814	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
580625	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
811449	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	not assigned
866365	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Caulobacter*	not assigned
849118	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
573013	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
New.0.CleanUp.ReferenceOTU4830	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
1072673	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
166064	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	not assigned
989109	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
1109964	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
136151	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
343503	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned

573258	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.0.CleanUp.ReferenceOTU8268	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.1.CleanUp.ReferenceOTU2902	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
620684	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
547097	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
571263	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
4396454	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
689696	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
583766	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
672144	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU3919	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU7272	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.1.CleanUp.ReferenceOTU2316	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
210344	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
279358	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
New.0.CleanUp.ReferenceOTU8673	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
345333	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Phyllobacterium*	not assigned
211171	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Polaromonas*	not assigned
3314521	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
252345	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Pseudoxanthomonas*	not assigned
New.0.CleanUp.ReferenceOTU4429	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ramlibacter*	not assigned
New.1.CleanUp.ReferenceOTU1726	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ramlibacter*	not assigned
222792	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
358785	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
317632	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
545247	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
616682	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
1638797	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
210914	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.0.CleanUp.ReferenceOTU4241	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned

New.0.CleanUp.ReferenceOTU9193	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU99	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU1979	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU802	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.1.CleanUp.ReferenceOTU915	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
1003206	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
New.0.CleanUp.ReferenceOTU10354	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
805685	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	not assigned
4395040	Acidobacteria	Solibacteres	Solibacterales	[Bryobacteraceae]	not assigned	not assigned
New.0.CleanUp.ReferenceOTU1714	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	not assigned	not assigned
1015820	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	not assigned	not assigned
363617	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
1068155	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU3451	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5301	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6720	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU274	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5418	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5587	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU11073	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	not assigned	not assigned
544711	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6745	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
1027418	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU6303	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	not assigned	not assigned
962736	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	not assigned	not assigned
215375	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4459	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	not assigned	not assigned
709657	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
564004	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
810700	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned

831407	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
527397	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
4373617	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
775149	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
1106653	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU3078	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4846	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU4994	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU7347	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8889	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU1526	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU2721	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	not assigned	not assigned
917888	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8799	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU1501	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	not assigned	not assigned
945478	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned
222768	Proteobacteria	Alphaproteobacteria	Ellin329	not assigned	not assigned	not assigned
399818	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
698517	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
216418	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2144	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
113261	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
1021984	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU8025	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
800292	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
583294	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
216643	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
328181	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
818282	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	not assigned	not assigned
807804	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned

4395045	Acidobacteria	Solibacteres	Solibacterales	not assigned	not assigned	not assigned
113674	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
901995	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
1090634	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2451	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU9530	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	not assigned	not assigned
583489	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU9789	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
817874	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus solibacter*	not assigned
New.0.CleanUp.ReferenceOTU6614	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
331282	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.0.CleanUp.ReferenceOTU7994	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*Niabella*	not assigned
941803	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*	not assigned
976441	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	*Pedobacter*	not assigned
646549	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
398604	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
2025156	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
206752	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU10543	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU2630	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	not assigned	not assigned
809485	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
2063454	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5885	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	not assigned	not assigned
New.1.CleanUp.ReferenceOTU17	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	not assigned	not assigned
281360	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU5330	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	not assigned	not assigned
New.0.CleanUp.ReferenceOTU7237	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	not assigned	not assigned
578443	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	not assigned	not assigned
828254	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned

New.0.CleanUp.ReferenceOTU6557	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	not assigned	not assigned
576136	Proteobacteria	Alphaproteobacteria	Ellin329	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU1177	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU7598	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	not assigned	not assigned
547926	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	not assigned	not assigned
250522	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
584598	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	not assigned	not assigned
New.0.CleanUp.ReferenceOTU1969	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
331849	Proteobacteria	Betaproteobacteria	not assigned	not assigned	not assigned	not assigned
513222	Chlorobi	SJA-28	not assigned	not assigned	not assigned	not assigned
551999	Bacteroidetes	VC2_1_Bac22	not assigned	not assigned	not assigned	not assigned

Table S11

Bulk soil: Shared Core OTUs (May, June, October) high LUI: 206						
OTU ID	**Phylum**	**Class**	**Order**	**Family**	***Genus***	***Species***
545324	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*	*bowmanii*
1069076	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*Adhaeribacter*	not assigned
New.0.CleanUp.ReferenceOTU10984	Proteobacteria	Alphaproteobacteria	Rhizobiales	Rhodobiaceae	*Afifella*	not assigned
New.11.CleanUp.ReferenceOTU75	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Afipia*	not assigned
961922	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
838594	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
580703	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
681987	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Balneimonas*	not assigned
236922	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
4377104	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
580625	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573764	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
New.21.CleanUp.ReferenceOTU5246	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
98457	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
New.9.CleanUp.ReferenceOTU5637	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1105814	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
736463	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
New.4.CleanUp.ReferenceOTU3180	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
New.18.CleanUp.ReferenceOTU9884	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
New.3.CleanUp.ReferenceOTU7152	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
New.10.CleanUp.ReferenceOTU18200	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
136297	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned

817874	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
576059	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
849118	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
New.0.CleanUp.ReferenceOTU8431	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
1072673	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
New.17.CleanUp.ReferenceOTU1700	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
701911	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	*Geobacter*	not assigned
New.4.CleanUp.ReferenceOTU9070	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.3.CleanUp.ReferenceOTU80	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.10.CleanUp.ReferenceOTU4474	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.0.CleanUp.ReferenceOTU8268	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.3.CleanUp.ReferenceOTU6571	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.2.CleanUp.ReferenceOTU4934	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
331282	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.1.CleanUp.ReferenceOTU2902	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
343503	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
136151	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
547097	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
571263	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
620684	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
New.7.CleanUp.ReferenceOTU7143	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
New.5.CleanUp.ReferenceOTU9623	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.1.CleanUp.ReferenceOTU2316	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.10.CleanUp.ReferenceOTU5569	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU7272	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU7828	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.4.CleanUp.ReferenceOTU5167	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
583766	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
672144	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned

173004	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	*Nitrospira*	not assigned
New.22.CleanUp.ReferenceOTU21670	Proteobacteria	Betaproteobacteria	A21b	UD5	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU3451	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU6050	Proteobacteria	Betaproteobacteria	A21b	UD5	*not assigned*	not assigned
945478	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*not assigned*	not assigned
818282	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	*not assigned*	not assigned
223126	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
737110	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*not assigned*	not assigned
205900	Acidobacteria	Solibacteres	Solibacterales	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU7347	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
1068155	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU3175	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU2721	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
539336	Acidobacteria	PAUC37f	not assigned	not assigned	*not assigned*	not assigned
New.2.CleanUp.ReferenceOTU2676	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU38	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
204335	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU8554	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU5964	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
511074	Acidobacteria	Solibacteres	Solibacterales	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU274	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*not assigned*	not assigned
New.22.CleanUp.ReferenceOTU17856	Acidobacteria	Solibacteres	Solibacterales	[Bryobacteraceae]	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU9530	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
142384	Proteobacteria	Betaproteobacteria	A21b	UD5	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU6011	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	*not assigned*	not assigned
New.7.CleanUp.ReferenceOTU6188	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4763	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
813632	Proteobacteria	Deltaproteobacteria	Myxococcales	OM27	*not assigned*	not assigned
1044319	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
4395040	Acidobacteria	Solibacteres	Solibacterales	[Bryobacteraceae]	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2421	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned

New.5.CleanUp.ReferenceOTU5805	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*not assigned*	not assigned
966576	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
New.23.CleanUp.ReferenceOTU3592	Acidobacteria	Solibacteres	Solibacterales	not assigned	*not assigned*	not assigned
New.6.CleanUp.ReferenceOTU1636	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.7.CleanUp.ReferenceOTU1208	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU8799	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU10526	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned
4395045	Acidobacteria	Solibacteres	Solibacterales	not assigned	*not assigned*	not assigned
328181	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	*not assigned*	not assigned
854368	Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	*not assigned*	not assigned
131337	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU1501	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*not assigned*	not assigned
New.9.CleanUp.ReferenceOTU981	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU4846	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
243855	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4994	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4981	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU5687	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
962736	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	*not assigned*	not assigned
698517	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
215375	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
4373617	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2355	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU14172	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
775149	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.22.CleanUp.ReferenceOTU8625	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU9635	Proteobacteria	Gammaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU1940	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
4424338	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
583489	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU5301	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned

New.2.CleanUp.ReferenceOTU1387	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.7.CleanUp.ReferenceOTU7457	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
206752	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU3936	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU4583	Proteobacteria	Gammaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.2.CleanUp.ReferenceOTU6790	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
576136	Proteobacteria	Alphaproteobacteria	Ellin329	not assigned	*not assigned*	not assigned
759507	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU9789	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2144	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2451	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
New.24.CleanUp.ReferenceOTU21377	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
254098	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
1044436	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.19.CleanUp.ReferenceOTU5725	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.12.CleanUp.ReferenceOTU1153	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
113261	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	*not assigned*	not assigned
New.14.CleanUp.ReferenceOTU265	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU8119	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.14.CleanUp.ReferenceOTU6502	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
800292	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU1945	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
807804	Acidobacteria	Solibacteres	Solibacterales	not assigned	*not assigned*	not assigned
539907	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU855	Firmicutes	Bacilli	Bacillales	Bacillaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU6745	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU8013	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	*not assigned*	not assigned
564004	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.2.CleanUp.ReferenceOTU5595	Firmicutes	Bacilli	Bacillales	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU3078	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
250522	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned

New.0.CleanUp.ReferenceOTU2877	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU2638	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*not assigned*	not assigned
113674	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
216418	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU1526	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
539866	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	*not assigned*	not assigned
New.18.CleanUp.ReferenceOTU3336	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*not assigned*	not assigned
1027418	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	*not assigned*	not assigned
831407	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.23.CleanUp.ReferenceOTU3077	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4459	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
New.22.CleanUp.ReferenceOTU6952	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
513222	Chlorobi	SJA-28	not assigned	not assigned	*not assigned*	not assigned
751555	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.7.CleanUp.ReferenceOTU7401	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
819885	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	*not assigned*	not assigned
527397	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
1090634	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU2121	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.6.CleanUp.ReferenceOTU1268	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
1111437	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
591683	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
207866	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
210344	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.0.CleanUp.ReferenceOTU2136	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
203611	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.10.CleanUp.ReferenceOTU16521	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.2.CleanUp.ReferenceOTU211	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.16.CleanUp.ReferenceOTU3113	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
279358	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
345333	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Phyllobacterium*	not assigned

New.6.CleanUp.ReferenceOTU1087	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.6.CleanUp.ReferenceOTU4928	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Ramlibacter*	not assigned
253167	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.7.CleanUp.ReferenceOTU5146	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU2542	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.2.CleanUp.ReferenceOTU6889	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
1638797	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU1979	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
358785	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
210914	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU2305	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU4519	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.2.CleanUp.ReferenceOTU3236	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU1675	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.7.CleanUp.ReferenceOTU4598	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.10.CleanUp.ReferenceOTU14995	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
317632	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU99	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU3855	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
2025156	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.12.CleanUp.ReferenceOTU6900	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
616682	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
545247	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.0.CleanUp.ReferenceOTU4241	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
222792	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU8738	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.1.CleanUp.ReferenceOTU802	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.1.CleanUp.ReferenceOTU915	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
1003206	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
New.0.CleanUp.ReferenceOTU3375	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
805685	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Thermomonas*	not assigned

Table S12

Bulk soil: Shared Core OTUs (May, June, October) low LUI: 220						
OTU ID	**Phylum**	**Class**	**Order**	**Family**	***Genus***	***Species***
1052559	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	*asaccharolytica*
545324	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*Clostridium*	*bowmanii*
New.10.CleanUp.ReferenceOTU12056	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*umsongensis*
1638797	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.11.CleanUp.ReferenceOTU75	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Afipia*	not assigned
961922	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
838594	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
580703	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
New.15.CleanUp.ReferenceOTU7158	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
New.8.CleanUp.ReferenceOTU8671	Firmicutes	Bacilli	Bacillales	Bacillaceae	*Bacillus*	not assigned
681987	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Balneimonas*	not assigned
4377104	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
580625	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573764	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
New.21.CleanUp.ReferenceOTU5246	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
98457	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
New.9.CleanUp.ReferenceOTU5637	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
1105814	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
573135	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*Bradyrhizobium*	not assigned
New.10.CleanUp.ReferenceOTU18200	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
136297	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
817874	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
576059	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	*Candidatus Solibacter*	not assigned
849118	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
New.0.CleanUp.ReferenceOTU4830	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Devosia*	not assigned
1072673	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
New.17.CleanUp.ReferenceOTU1700	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned

New.0.CleanUp.ReferenceOTU6614	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	*Flavobacterium*	not assigned
New.0.CleanUp.ReferenceOTU8268	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.3.CleanUp.ReferenceOTU6571	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.2.CleanUp.ReferenceOTU4934	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
331282	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.1.CleanUp.ReferenceOTU2902	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
343503	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
136151	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
New.0.CleanUp.ReferenceOTU6126	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
1109964	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Kaistobacter*	not assigned
543156	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Labrys*	not assigned
547097	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
571263	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
620684	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
New.7.CleanUp.ReferenceOTU7143	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
New.26.CleanUp.ReferenceOTU2077	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Mesorhizobium*	not assigned
New.0.CleanUp.ReferenceOTU7828	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.4.CleanUp.ReferenceOTU5167	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
583766	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
672144	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
4396454	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
226906	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.22.CleanUp.ReferenceOTU24740	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.0.CleanUp.ReferenceOTU1365	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.10.CleanUp.ReferenceOTU10097	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Methylibium*	not assigned
New.8.CleanUp.ReferenceOTU4846	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
243855	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4994	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4981	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU5687	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
962736	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	*not assigned*	not assigned

698517	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
215375	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
4373617	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2355	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU14172	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
775149	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.22.CleanUp.ReferenceOTU8625	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU9635	Proteobacteria	Gammaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU1940	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
4424338	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
583489	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU5301	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.2.CleanUp.ReferenceOTU1387	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.7.CleanUp.ReferenceOTU7457	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
206752	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU3936	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU4583	Proteobacteria	Gammaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.2.CleanUp.ReferenceOTU6790	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
576136	Proteobacteria	Alphaproteobacteria	Ellin329	not assigned	*not assigned*	not assigned
759507	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU9789	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2144	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2451	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
New.24.CleanUp.ReferenceOTU21377	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
254098	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
1044436	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.19.CleanUp.ReferenceOTU5725	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.12.CleanUp.ReferenceOTU1153	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
113261	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	*not assigned*	not assigned
New.14.CleanUp.ReferenceOTU265	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU8119	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned

New.14.CleanUp.ReferenceOTU6502	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
800292	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU1945	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
807804	Acidobacteria	Solibacteres	Solibacterales	not assigned	*not assigned*	not assigned
539907	Proteobacteria	Deltaproteobacteria	Syntrophobacterales	Syntrophobacteraceae	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU855	Firmicutes	Bacilli	Bacillales	Bacillaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU6745	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU8013	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	*not assigned*	not assigned
564004	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.2.CleanUp.ReferenceOTU5595	Firmicutes	Bacilli	Bacillales	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU3078	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
250522	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2877	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*not assigned*	not assigned
New.8.CleanUp.ReferenceOTU2638	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*not assigned*	not assigned
113674	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
216418	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU1526	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
539866	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	*not assigned*	not assigned
New.18.CleanUp.ReferenceOTU3336	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	*not assigned*	not assigned
1027418	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Erythrobacteraceae	*not assigned*	not assigned
831407	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.23.CleanUp.ReferenceOTU3077	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4459	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
New.22.CleanUp.ReferenceOTU6952	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
513222	Chlorobi	SJA-28	not assigned	not assigned	*not assigned*	not assigned
751555	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.7.CleanUp.ReferenceOTU7401	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*not assigned*	not assigned
819885	Proteobacteria	Gammaproteobacteria	HTCC2188	HTCC2089	*not assigned*	not assigned
527397	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
1090634	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU2121	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned

New.6.CleanUp.ReferenceOTU1268	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
1111437	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU230	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU1791	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU21512	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
758928	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	*not assigned*	not assigned
New.26.CleanUp.ReferenceOTU24479	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.14.CleanUp.ReferenceOTU3008	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.3.CleanUp.ReferenceOTU4159	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
832626	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	*not assigned*	not assigned
New.26.CleanUp.ReferenceOTU624	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4846	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.24.CleanUp.ReferenceOTU22461	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU490	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU10762	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.5.CleanUp.ReferenceOTU2437	Proteobacteria	Gammaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
399818	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.25.CleanUp.ReferenceOTU10034	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
547926	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	*not assigned*	not assigned
4339472	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*not assigned*	not assigned
709657	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.25.CleanUp.ReferenceOTU27075	Proteobacteria	Betaproteobacteria	not assigned	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU2357	Proteobacteria	Betaproteobacteria	SC-I-84	not assigned	*not assigned*	not assigned
241220	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	*not assigned*	not assigned
New.4.CleanUp.ReferenceOTU11338	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU5885	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Hyphomonadaceae	*not assigned*	not assigned
859313	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	*not assigned*	not assigned
New.13.CleanUp.ReferenceOTU9811	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*not assigned*	not assigned
1008533	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	*not assigned*	not assigned
540592	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	*not assigned*	not assigned
542933	Actinobacteria	Acidimicrobiia	Acidimicrobiales	EB1017	*not assigned*	not assigned

New.23.CleanUp.ReferenceOTU12116	Proteobacteria	Alphaproteobacteria	Rhizobiales	not assigned	*not assigned*	not assigned
New.10.CleanUp.ReferenceOTU25647	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU2728	Actinobacteria	Actinobacteria	Actinomycetales	not assigned	*not assigned*	not assigned
566368	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.17.CleanUp.ReferenceOTU1263	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU1714	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*not assigned*	not assigned
New.26.CleanUp.ReferenceOTU11842	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.26.CleanUp.ReferenceOTU23089	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*not assigned*	not assigned
New.16.CleanUp.ReferenceOTU7478	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
281360	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	*not assigned*	not assigned
New.25.CleanUp.ReferenceOTU10163	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU1371	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	*not assigned*	not assigned
4302753	Proteobacteria	Deltaproteobacteria	Myxococcales	Cystobacteraceae	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU4683	Proteobacteria	Betaproteobacteria	Ellin6067	not assigned	*not assigned*	not assigned
New.1.CleanUp.ReferenceOTU2248	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU505	Proteobacteria	Deltaproteobacteria	Myxococcales	not assigned	*not assigned*	not assigned
New.0.CleanUp.ReferenceOTU8454	Proteobacteria	Deltaproteobacteria	[Entotheonellales]	[Entotheonellaceae]	*not assigned*	not assigned
1051517	Firmicutes	Bacilli	Bacillales	not assigned	*not assigned*	not assigned
901995	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	not assigned	*not assigned*	not assigned
207866	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
210344	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.0.CleanUp.ReferenceOTU2136	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
203611	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.10.CleanUp.ReferenceOTU16521	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.2.CleanUp.ReferenceOTU211	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.16.CleanUp.ReferenceOTU3113	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
New.25.CleanUp.ReferenceOTU615	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Pedomicrobium*	not assigned
279358	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
New.1.CleanUp.ReferenceOTU1050	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
New.0.CleanUp.ReferenceOTU8673	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Phenylobacterium*	not assigned
345333	Proteobacteria	Alphaproteobacteria	Rhizobiales	Phyllobacteriaceae	*Phyllobacterium*	not assigned

New.6.CleanUp.ReferenceOTU1087	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
3314521	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.5.CleanUp.ReferenceOTU9448	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	not assigned
New.1.CleanUp.ReferenceOTU1979	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
358785	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
210914	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU2305	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU4519	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.2.CleanUp.ReferenceOTU3236	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU1675	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.7.CleanUp.ReferenceOTU4598	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.10.CleanUp.ReferenceOTU14995	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
317632	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU99	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.3.CleanUp.ReferenceOTU3855	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
2025156	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.12.CleanUp.ReferenceOTU6900	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
616682	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
545247	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.0.CleanUp.ReferenceOTU4241	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
222792	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU6595	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.4.CleanUp.ReferenceOTU1289	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.0.CleanUp.ReferenceOTU9193	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	*Rhodoplanes*	not assigned
New.1.CleanUp.ReferenceOTU802	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.1.CleanUp.ReferenceOTU915	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
New.0.CleanUp.ReferenceOTU9179	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	*Rubrivivax*	not assigned
555945	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	*SMB53*	not assigned
1003206	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
New.0.CleanUp.ReferenceOTU10354	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned
New.5.CleanUp.ReferenceOTU3637	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	not assigned

New.0.CleanUp.ReferenceOTU3375	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
New.16.CleanUp.ReferenceOTU180	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
New.1.CleanUp.ReferenceOTU1422	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
New.2.CleanUp.ReferenceOTU2537	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
New.0.CleanUp.ReferenceOTU10336	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
New.22.CleanUp.ReferenceOTU24001	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned
New.13.CleanUp.ReferenceOTU6645	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	*Steroidobacter*	not assigned