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From leaf to soil —
Nitrogen partitioning and stabilization in soil organic matter

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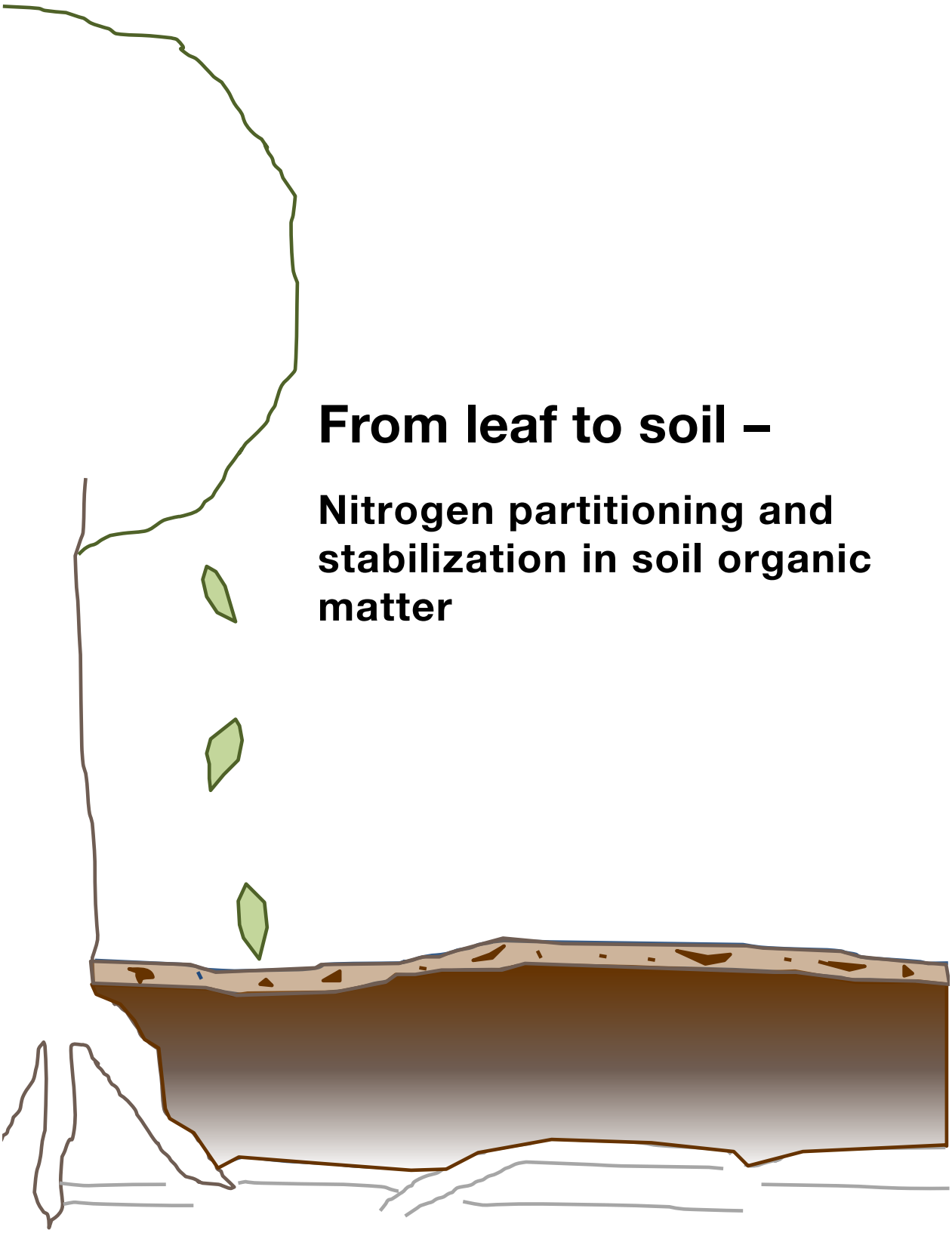

Beech forest at the research site near Tuttlingen in space and time

Summary

European beech forests stocking on marginal nitrogen-poor soils are widespread across Europe offering economic importance and ecosystem value. However, these ecosystems are susceptible to water deprivation predicted for the 21st century.

The focus of this research is the elucidation of major nitrogen stabilization processes in soil organic matter as part of the nitrogen competition between stabilization and bioavailability in the beech-soil system of a broadleaf forest in Central Europe. The approach contained two field studies (**Study I** and **II**) which assessed nitrogen stabilization and turnover by using ^{15}N isotope labeling and a laboratory experiment (**Study III**) which investigated the mineralization behavior of particle size fractions. These investigations provided comprehensive data identifying specific nitrogen fractions and their turnover under the influence of climatic impacts induced by climate change. ^{15}N labeling whether in an organic or inorganic form was an important tool to study nitrogen cycling processes in the soil within this project. It allowed tracing the fate of nitrogen through different soil fractions in both field experiments (**Study I** and **II**) and thus, to quantify the partitioning of nitrogen between plants, microbial biomass and different soil compartments in the field. To obtain the different soil organic matter fractions from the bulk soil, a density fractionation scheme was applied using sodium polytungstate adapted to the soil of the experiments.

Study I presents the results obtained from a 3-year field experiment using ^{15}N-labeled beech leaf litter (*Fagus sylvatica* L.) in replacement of the natural litter layer of a broadleaf forest soil. A combined soil particle size and density fractionation procedure was applied to describe the decomposition and stabilization of litter-derived nitrogen. This study closes the gap for the transfer of litter-derived nitrogen at the initial stage of litter decay over the first years after application in situ. The fate of recently applied nitrogen was followed in tight time-scales. ^{15}N storage in soil fractions demonstrated that the clay fractions acted as the main sink for nitrogen, with an unexpectedly rapid enrichment of nearly three percent of the added ^{15}N excess from labeled litter into the

clay fractions within 140 days. This result highlights the dominant role of the clay fractions in the stabilization of nitrogen in the investigated soil. A delayed translocation of the ^{15}N tracer in the occluded light fractions was observed, whereas the dynamics of ^{15}N in the free light fractions could be traced back to leaching effects and the high bioturbation at the study site.

Study II analyzed the potential climate change induced drought effects on nitrogen cycling and the partitioning of nitrogen among soil fractions under a European beech forest (*Fagus sylvatica* L.). A stable isotope-based experimental approach to simultaneously quantify all major nitrogen turnover processes in intact beech-soil-microbe-mesocosms, thereby maintaining plant-soil-microbe interactions and competition for nitrogen was deployed. By translocation of undisturbed soil mesocosms containing natural beech regeneration across a narrow valley from the cool-moist northwest to the warm-dry southwest aspect, this labeling approach was combined with a space-for-time climate change experiment. This treatment manipulated soil temperature on average by +1 °C and persistently reduced soil moisture (reduction of up to 40% of volumetric soil water content) over the entire growing season intensified by a temporary rain-shelter. Subsequently, a ^{15}N labeling ($^{15}NH_4{}^{15}NO_3$ at 99 atom% ^{15}N enrichment) via 40 injections plus a surface label application per soil column guaranteed a homogenous spreading of the tracer within the mesocosm, which was followed by destructive sampling on days 0, 7, 32, and 97. A combined soil particle size and density fractionation procedure revealed greater ^{15}N concentrations in light fractions under drought indicating lower stability. On the other side, proportionally less nitrogen was bound in organo-mineral associations of the drought-treated mesocosms compared to the controlled references. This was ascribed to a less active microbial community. Hence, prolonged summer droughts may alter nitrogen cycling and subsequently, its stabilization. This could lead to nitrogen loss under future climate change scenarios due to a retarded stabilization.

To verify the stabilization of soil organic matter within different fractions, a mineralization experiment was simulated (**Study III**) and associated with the field trials.

The scope of this laboratory incubation was to quantify the bioavailability of carbon and nitrogen in different particle size fractions in order to develop an understanding of soil carbon and nitrogen stabilization patterns in specific soil organic matter fractions. The chemical composition of selected samples was further characterized by ^{13}C-NMR spectroscopy. Differences between fractions and the bulk soil elucidated the relative significance and role of the fractions in the soil as a whole. The results demonstrated that carbon mineralization was not influenced by fractionation, whereas for nitrogen mineralization, the mathematically recombined fractions had significantly lower mineralization rates than the bulk soil. In the sand fraction, which comprised a litter-like particulate organic matter fraction, carbon was bioavailable for mineralization, but nitrogen was limited. The comparably high mineralization rate of the clay fraction at the beginning of the experiment was attributed to the labile pool transferred to this fine grain size via the fractionation procedure. During the remainder of the experiment, the clay fraction's nitrogen immobilization was inhibited due to carbon limitation. To conclude, **Study III** confirmed that soil organic matter bioavailability controlled the effectiveness of carbon and nitrogen mineralization. It is known that carbon and nitrogen mineralization are closely coupled processes during the decomposition of plant residues on or in the soil. **Study III** suggests that they are decoupled in the mineral-associated fractions of the soil, as the interactions of both carbon and nitrogen containing components with the mineral matrix strongly modulate the mineralization dynamics. **Study III** showed that C/N and alkyl C to O/N-alkyl C ratios of organic matter are not appropriate to describe bioavailability and biological decomposability in fractions where organic matter is mainly stabilized by spatial inaccessibility and by organo-mineral interactions.

In summary, this thesis considering the three independent investigations showed that in clay-rich soils, such as at Tuttlingen, the formation of organo-mineral associations features prominently the stabilization of nitrogen. The clay fractions act as the main sink for nitrogen, but also as a source for nitrogen mineralization. Light fractions serve as a transitory pool with short-term passage for nitrogen and are reliant on aggregate turnover. With a higher frequency of extended summer droughts in the course of

climate change in Central Europe, stabilization patterns will change: Nitrogen stabilization in organo-mineral associations will be decelerated and nitrogen will stay longer in more labile fractions, always at the risk of leaching losses under predicted heavy precipitation events. The mineralization experiment discovered that the bioavailability of soil organic matter controls the effectiveness of carbon and nitrogen mineralization. The data further revealed that the C/N ratio as a key performance indicator for biological decomposability, which is often used to distinguish humus forms of the litter layer, is not appropriate to characterize the clay fraction.

Zusammenfassung

Die Verbreitung von Buchenwäldern auf marginalen und stickstoffarmen Standorten erstreckt sich über ganz Europa und ist von ökonomischer und ökologischer Bedeutsamkeit. Diese Ökosysteme sind allerdings anfällig gegenüber Wassermangel, der für das 21. Jahrhundert prognostiziert wird.

Zentraler Forschungsgegenstand dieser Arbeit war die Aufklärung der wichtigsten Stickstoffstabilisierungsprozesse in organischer Bodensubstanz in einem Buchen-Boden System eines zentraleuropäischen Laubwaldes im Zuge der Stickstoffkonkurrenz zwischen Stabilisierung und Bioverfügbarkeit. Die Herangehensweise beinhaltet zwei Feldstudien (**Studie I** und **II**), die die Stickstoffstabilisierung und den Umsatz mit Hilfe von ^{15}N Stabilisotopenmarkierungs-technik evaluieren sowie ein Laborexperiment (**Studie III**), mit dem Ziel das Mineralisierungsverhalten von Korngrößenfraktionen beurteilen zu können. Diese Untersuchungen liefern einen umfassenden Datensatz, der spezifische Stickstofffraktionen und ihren Umsatz im Hinblick auf klimatische Auswirkungen des Klimawandels näher betrachtet. Die Markierung mit dem schweren Stickstoffisotop ^{15}N in organischer und anorganischer Form diente hierbei als wichtige Methode um den Stickstoffkreislauf im Boden zu untersuchen. Dieser Ansatz ermöglichte es den Verbleib des applizierten Stickstoffs durch die verschiedenen Bodenfraktionen in den beiden Freilandversuchen (**Studie I** und **II**) zu verfolgen und damit die Stickstoffverteilung zwischen Pflanzen, mikrobieller Biomasse und den verschieden Bodenkomponenten zu quantifizieren. Um die verschiedenen Bodenfraktionen aus dem Gesamtboden zu gewinnen, wurde ein Dichtefraktionierungsschema mittels Natriumpolywolframat angewandt, das an den spezifischen Boden der Experimente angepasst wurde.

Studie I präsentiert die Ergebnisse eines dreijährigen Feldexperimentes, die mit Hilfe von ^{15}N markierter Buchenstreu (*Fagus sylvatica* L.) als Ersatz für die natürliche Streuauflage in einem Boden unter Laubwald gewonnen wurden. Eine kombinierte Partikelgrößen- und Dichtefraktionierung wurde angewandt, um die Zersetzung und Stabilisierung von streubürtigem Stickstoff zu beschreiben. Diese Studie schließt die

Lücke für den Transfer von streubürtigem Stickstoff während der initialen Stufe des Streuabbaus in den ersten Jahren nach einer in situ Aufbringung. Der Verbleib des kürzlich aufgebrachten Stickstoffs wurde in kurzen Zeitintervallen verfolgt. Die ^{15}N Speicherung in den Bodenfraktionen zeigte, dass die Tonfraktionen sich als größte Stickstoffsenke verhielten, die durch eine unerwartet schnelle Anreicherung von drei Prozent der aufgebrachten ^{15}N excess Markierung innerhalb von 140 Tagen gekennzeichnet waren. Dieses Ergebnis unterstreicht die dominante Rolle der Tonfraktionen im untersuchten Boden während des Stickstoffstabilisierungsprozesses. Eine verzögerte Translokation des ^{15}N Tracers in die okkludierte leichte Fraktion wurde beobachtet, während die Dynamik des ^{15}N in der freien leichten Fraktion auf Auswaschungseffekte und die hohe Bioturbation am Standort zurückzuführen ist.

Studie II analysierte die potentiellen, durch den Klimawandel induzierten Trockenheitseffekte auf den Stickstoffkreislauf und die Verteilung von Stickstoff in den Bodenfraktionen in einem europäischen Buchenwald (*Fagus sylvatica* L.). Ein angereicherter auf Stabilisotopentechnik basierender experimenteller Ansatz wurde benutzt, um alle wichtigen Prozesse des Stickstoffkreislaufes in intakten Buchen-Boden-Mikroorganismen-Mesokosmen zu quantifizieren, wobei die Interaktionen und die Konkurrenz um Stickstoff zwischen Pflanze, Boden und Mikoorganismen aufrechterhalten blieben. Zu dieser Herangehensweise wurde ein „space-for-time“ Versetzungsexperiment kombiniert, bei dem intakte Bodenmesokosmen mit Jungbuchen eines kühl-feuchten Nordwesthangs auf den gegenüberliegenden warm-trockenen Südwesthang transferiert wurden, wo sie erhöhter Temperatur (+1 °C) und geringerer Bodenfeuchtigkeit (Reduktion um bis zu 40% des volumetrischen Wassergehalts) ausgesetzt wurden. Diese mesoklimatische Veränderung wurde durch ein temporäres Regendach verstärkt. Anschließend wurde die ^{15}N Markierung ($^{15}NH_4{}^{15}NO_3$ bei 99 atom% ^{15}N Anreicherung) mittels 40 Injektionen und einer Oberflächenmarkierung pro Bodensäule appliziert, was eine homogene Verteilung des Markierungsstoffes im Mesokosmos garantierte. Eine destruktive Beprobung erfolgte nach 0, 7, 32 und 97 Tagen. Eine kombinierte Partikel- und Dichtefraktionierung zeigte eine höhere ^{15}N Konzentration in den leichten Fraktionen unter Trockenheit, was einer

geringeren Stabilisierung entspricht. Auf der anderen Seite wurde im Vergleich zu den Mesokosmen der Referenzkontrolle weniger Stickstoff in organo-mineralischen Assoziationen in den Mesokosmen unter Trockenheit gebunden. Dies steht im Zusammenhang mit einer weniger aktiven mikrobiellen Gemeinschaft. Daher kann verlängerte Sommertrockenheit den Stickstoffkreislauf und folglich die Stabilisierung modifizieren. Diese verzögerte Stabilisierung könnte zu Stickstoffverlusten unter den prognostizierten Klimaszenarien führen.

Um die Stabilisierung der organischen Bodensubstanz in verschiedenen Fraktionen zu überprüfen, wurde ein Mineralisierungsexperiment angeschlossen (**Studie III**). Das Ziel dieser Laborinkubation war eine Quantifizierung der Bioverfügbarkeit von Kohlenstoff und Stickstoff in verschiedenen Korngrößenfraktionen, um ein besseres Verständnis für Kohlenstoff- und Stickstoffstabilisierungsmuster in spezifischen Fraktionen der organischen Bodensubstanz zu entwickeln. Die chemische Zusammensetzung von ausgewählten Proben wurde durch ^{13}C-NMR-Spektroskopie charakterisiert. Unterschiede zwischen Fraktionen und Gesamtboden verdeutlichten die relative Signifikanz und Rolle der Fraktionen innerhalb des Gesamtbodens. Die Ergebnisse zeigten, dass die Kohlenstoffmineralisierung nicht von der Fraktionierungsmethode beeinflusst wurde, während die Stickstoffmineralisierung bei der mathematischen Rekombination der Fraktionen eine signifikant niedrigere Mineralisierungsrate im Vergleich zum Gesamtboden zeigte. In der Sandfraktion, die eine auflagenähnliche Fraktion partikulärer organischer Substanz umfasste, war Kohlenstoff zur Mineralisierung verfügbar, allerdings war hier der Stickstoff limitiert. Die vergleichsweise hohe Mineralisierungsrate der Tonfraktion zu Beginn des Experimentes kann auf den labilen Pool zurückgeführt werden, der durch das Fraktionierungsverfahren in die feinen Korngrößen gespült wurde. Im weiteren Verlauf des Experimentes wurde die Stickstoffimmobilisierung des Tons durch eine Kohlenstofflimitierung behindert. Schlussfolgernd bestätigte **Studie III**, dass die Bioverfügbarkeit der organischen Bodensubstanz die Effektivität der Kohlenstoff- und Stickstoffmineralisierung kontrolliert. Es ist bekannt, dass die Prozesse der Kohlenstoff- und Stickstoffmineralisierung während der Zersetzung von Pflanzenrückständen auf oder im

Boden eng verbunden sind. **Studie III** hingegen weist auf eine Entkopplung dieser Prozesse in mineralgebundenen Bodenfraktionen hin, da die Interaktionen der kohlenstoff- und stickstoffhaltigen Komponenten mit der Mineralmatrix die Mineralisierungsdynamik stark beeinflussen. **Studie III** zeigte, dass das C/N- und das alkyl C zu O/N-alkyl C-Verhältnis der organischen Substanz nicht geeignet ist, um die Bioverfügbarkeit und biologische Zersetzbarkeit in Fraktionen zu erklären, in denen die Stabilisierung organischer Substanz hauptsächlich durch räumliche Unzugänglichkeit und organo-mineralische Verbindungen gegeben ist.

Zusammenfassend zeigte diese Arbeit basierend auf den drei Untersuchungen, dass in tonreichen Böden, wie sie bei Tuttlingen vorkommen, die Bildung von organo-mineralischen Verbindungen für die Stickstoffstabilisierung wichtig sind. Die Tonfraktionen fungieren als wichtigste Senke für Stickstoff, aber auch als Quelle für die Stickstoffmineralisierung. Die leichten Fraktionen dienen als kurzzeitiger Durchgangspool für Stickstoff und sind abhängig vom Aggregatumsatz. Mit einem häufigeren Auftreten von ausgedehnten sommerlichen Trockenperioden im Zuge des Klimawandels in Zentraleuropa werden sich die Stabilisierungsmuster ändern: Die Stickstoffstabilisierung in organo-mineralischen Assoziationen wird sich verlangsamen und Stickstoff wird länger in den labileren Fraktionen verbleiben, verbunden mit dem latenten Risiko der Auswaschung während prognostizierter Starkregenereignisse. Das Mineralisierungsexperiment stellte fest, dass die Bioverfügbarkeit von organischer Bodensubstanz die Effektivität der Kohlenstoff- und Stickstoffmineralisierung kontrolliert. Der Datensatz zeigte weiter, dass das C/N-Verhältnis, das oft zur Unterscheidung von Auflagehumusformen herangezogen wird, als Hauptmerkmal für biologische Zersetzbarkeit der Tonfraktion ungeeignet ist.

Thesis at a glance

List of publications and contributions

1. Publications

This scientific doctoral dissertation is based on the following research articles:

Study I: **Bimüller C**, Naumann P, Buegger F, Dannenmann F, Zeller B, von Lützow M, Kögel-Knabner I (2013): Rapid transfer of 15N from labeled beech leaf litter to functional soil organic matter fractions in a Rendzic Leptosol. Soil Biology and Biochemistry 58, 323–331.

Study II: **Bimüller C**, Dannenmann M, Tejedor J, von Lützow M, Buegger F, Meier R, Haug S, Schroll R, Kögel-Knabner I (2014): Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions. Soil Biology and Biochemistry 68, 241–251.

Study III: **Bimüller C**, Mueller C W, von Lützow M, Kreyling O, Kölbl A, Haug, S, Schloter M, Kögel-Knabner I (2014): Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil. Soil Biology and Biochemistry 78, 263–273.

The published manuscripts are attached in the appendix.

Other related co-authored research articles from joint experiments by the Beech Research Group FOR 788 and the follow-up joint proposal under the contract numbers DA 1217/2-1, KO 1035/41-1, SCHL 446/11-1, RE 515/33-1 and PO 362/19-1:

Study IV: Gschwendtner S, Tejedor J, **Bimüller C**, Dannenmann M, Kögel-Knabner I, Schloter M (2014): Climate change induces shifts in microbial nitrogen cycling towards mineralization and incomplete denitrification in the soil of a Mid-European beech forest. PLoSONE 9(12): e114278. doi:10.1371/journal.pone.0114278

Study V: Guo C, Simon J, Gasche R, Naumann P S, **Bimüller C**, Pena R, Polle A, Kögel-Knabner I, Zeller B, Rennenberg H, Dannenmann M (2013): Minor contribution of leaf litter to N nutrition of beech (Fagus sylvatica) seedlings in a mountainous beech forest of Southern Germany. Plant and Soil 369 (1-2): 657–668.

2. Contribution

My contribution to the papers listed in Study I-V:

Study I: I conducted field work, carried out laboratory analyses, evaluated the data and wrote the manuscript.

Study II: I was involved in designing the experiment, conducted field work, carried out laboratory analyses, evaluated the data and wrote the manuscript.

Study III: I established the idea, designed and performed the experiment, developed analytical approaches, collected and analyzed the data. I evaluated the data and wrote the manuscript.

Study IV: I participated in field work, carried out bulk soil analysis, gathered and provided the bulk soil data. I wrote the methods section about bulk soil analysis and commented on the whole manuscript.

Study V: I was involved in the development of the experimental design, participated in field work, prepared and analyzed bulk soil samples, provided bulk soil data and commented on the manuscript.

Study I — Rapid transfer of ^{15}N from labeled beech leaf litter to functional soil organic matter fractions in a Rendzic Leptosol

by Bimüller C, Naumann P, Buegger F, Dannenmann F, Zeller B, von Lützow M, Kögel-Knabner I

published in Soil Biology and Biochemistry 58, 323–331.

Aim To trace and quantify the stabilization of nitrogen released from litter decomposition in different functional soil organic matter fractions.

Methods ^{15}N-labeled beech litter was deposited on the bare soil surface of three 2 m × 2 m plots on a Rendzic Leptosol under beech (*Fagus sylvatica* L.) in Southern Germany. Different soil organic matter fractions were obtained by a combined density and particle size fractionation procedure and ^{15}N composition was monitored over three years.

Results Nitrogen from leaf litter typically decomposed in two phases. The first flush was detected already after 140 days, due to plant debris transferred to the free light fraction by probably bioturbation and soluble compounds being leached from the litter directly to the clay fractions. The translocation to the occluded light fractions was delayed owing to the aggregate turnover. Nine percent of added label were recovered after 876 days (0-10 cm depth).

Conclusions Clay fractions act as main sink for recovered ^{15}N playing the key role for nitrogen stabilization in organo-mineral associations besides protection of soil organic matter inside soil aggregates in the investigated soil.

Study II	Prolonged summer droughts retard soil N processing and stabilization

by Bimüller C, Dannenmann M, Tejedor J, von Lützow M, Buegger F, Meier R, Haug S, Schroll R, Kögel-Knabner I

published in Soil Biology and Biochemistry 68, 241–251.

Aim To explore the impact of prolonged summer droughts in the course of climate change on soil nitrogen processing, partitioning between and stabilization in organo-mineral fractions.

Methods A space-for-time climate change experiment transplanting intact plant–soil–microbe mesocosms across a valley from the cool moist site to the slope with a warm-dry microclimate was combined with a ^{15}N labeling approach using ammonium nitrate and a physical fractionation procedure as well as chemical soil extraction protocols.

Results Modified partitioning of recently applied inorganic ^{15}N between different soil fractions was observed, with attenuated nitrogen turnover under drought and consequently significantly higher ^{15}N concentrations in the relatively labile light fractions. This effect can be ascribed to a decelerated mineralization immobilization turnover. Within less than one growing season, drier soils accumulated more ^{15}N in the light fractions, functioning as a significant transitory, labile pool.

Conclusions Prolonged summer droughts may alter the stabilization dynamics because the induced inactivity of microorganisms may reduce the dislocation of nitrogen to stabilization pathways. A retarded stabilization in organo-mineral associations enhances the risk of nitrogen losses during extreme rainfall events.

Study III Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil

by Bimüller C, Mueller C W, von Lützow M, Kreyling O, Kölbl A, Haug, S, Schloter M, Kögel-Knabner I

published in Soil Biology and Biochemistry 78, 263–273.

Aim To identify the bioavailability for mineralization of carbon and nitrogen in the different particle size fractions and improve the understanding of the system concerning carbon and nitrogen dynamics in the soil.

Methods Topsoil was separated into particle size fractions. Bulk soil and fractions were incubated in replicates allowing periodic destructive sampling of random triplicates at days: 0, 14, 42, 84, 140, 210, and 280. We monitored CO_2-C respiration, NH_3-N emissions, nitrogen mineralization, carbon and nitrogen in microbial biomass and total carbon and nitrogen.

Results The destruction of the aggregated soil structure via fractionation did not influence carbon mineralization in spite of a higher accessibility and released salt extractable organic carbon, but decreased nitrogen mineralization. Soil organic matter bioavailability controlled the effectiveness of carbon and nitrogen mineralization. The microbial activity in the clay fraction was carbon limited and all carbon in this fraction was strongly bound to mineral surfaces. This was reverse in the sand fraction where nitrogen was limited, but carbon was available.

Conclusions Carbon and nitrogen mineralization are usually linked during decomposition processes, but are decoupled in the mineral-associated fractions of the soil, as the interactions of both with the mineral matrix strongly modulate the mineralization dynamics. Therefore, the C/N ratio as well as the alkyl C to O/N-alkyl C ratio as an indicator for biological decomposability is not useful when characterizing soil fractions that are

mainly stabilized by spatial inaccessibility and by organo-mineral interactions.

List of Figures

All photos are taken by me, Carolin Bimüller, if not otherwise stated.

List of Tables

Abbreviations

C	carbon
fLF	free light fraction
LF	light fraction
N	nitrogen
N_{min}	inorganic nitrogen corresponding mineral nitrogen
N_{tot}	total nitrogen
OC	organic carbon
oLF	occluded light fraction
ON	organic nitrogen
POM	particulate organic matter
SEOC	salt extractable organic carbon
SOM	soil organic matter
SPT	sodium polytungstate

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Content

1 Introduction

1.1 Background: European beech forests on calcareous soil are endangered by climate change

European beech forests (*Fagus sylvatica* L.) often stock on calcareous soils with shallow profiles and are widely disseminated in areas of sub-mountainous altitude in Central Europe (Ellenberg and Leuschner, 2010). These broadleaf ecosystems offer essential ecological services like microclimatic regulations, supporting nutrient cycles. They deliver recreational benefits and have a considerable economic importance. However, these marginal sites are known to have a low water retention capacity and are poor in bioavailable nitrogen (N) (Dannenmann et al., 2006; Geßler et al., 2007; Dannenmann et al., 2009), which is an essential nutrient for plant growth. Only an efficient microbial N turnover with low N losses via denitrification or leaching and rapid recycling of organically bound N by mineralization can supply a productive forest ecosystem (Schimel and Bennett, 2004). Moreover, these N-limited ecosystems are governed by a competition for N between microorganisms and plants (Dannenmann et al., 2009). The susceptibility of these ecosystems to projected climate change (Intergovernmental Panel on Climate Change IPCC, 2013) including higher temperatures, prolonged extreme summer droughts and extreme rainfall events is at present still uninvestigated. However, the apparent drought sensitivity of beech is currently under debate and of major concern (Leuschner et al., 2001; Rennenberg et al., 2006; Geßler et al., 2007; Rennenberg et al., 2009; Kreuzwieser and Gessler, 2010) due to observations of higher frequency of heat waves and droughts in wide regions of Central Europe (Coumou et al., 2013) leading to frequent Xeric (when soil is dry during most of the summer throughout the profile) periods (Trnka et al., 2013). It has been proposed that water deprivation may endorse N limitation of European beech due to impaired microbial N cycling in the soil, but this hypothesis has not yet been tested.

1.2 Research topic

Two main sources for bioavailable N in forest soils are litter decomposition and mineralization of soil organic matter (SOM). They feature prominently in forest nutrition as major pathways of ecosystem internal cycling. While litter decomposition itself is already well considered (Olson, 1963), little is known about the fate of released N within SOM fractions. These different SOM fractions can be isolated using fractionation methods and further be assessed regarding the stabilization pathways of SOM. Generally, competition for N in the beech-soil system is targeted on several N species (both organic and inorganic), involving several players (e.g. plants and microorganisms), and, furthermore, taking place via a wide range of both biotic and abiotic processes. Therefore, studying N dynamics comprises a complex methodological approach. Summer droughts induced by climate change as predicted for the coming decades (Intergovernmental Panel on Climate Change IPCC, 2013), will influence water tension and oxygen partial pressure in the soil. This will reduce the competitiveness of both, plant and soil microorganisms for N by changing the dynamics of N and water availability. The response of N partitioning between the fractions and the stabilization to these altered environmental conditions is unexplored. The various availabilities of N for mineralization and its link to carbon (C) mineralization in different particle size fractions have still not been studied in combination. This context is necessary to understand and judge the bioavailability of SOM.

The aim of this thesis was to elucidate the major stabilization processes for N in a Rendzic Leptosol (**Study I**) with special regard to leaf litter input and an assessment under climatically changing conditions (**Study II**). Different mineralization properties within particle size fractions were evaluated in connection with the link between C and N dynamics (**Study III**).

2 State of the art

2.1 Stabilization of soil organic matter

Stabilization of SOM has become a popular research question in soil science (Sollins et al., 1996; von Lützow et al., 2006; von Lützow et al., 2008). In temperate soils, three main stabilization mechanisms have been identified that protect SOM against decomposition: (1) different material properties which result in selective preservation of recalcitrant compounds besides (2) spatial inaccessibility and (3) interactions of SOM with minerals and metal ions (Schnitzer et al., 1978; Sollins et al., 1996; von Lützow et al., 2008). Different stages of SOM stability develop by the partly simultaneous operation of stabilization mechanisms (von Lützow et al., 2006). The role of selective preservation of recalcitrant SOM has recently been under debate (Kleber, 2010a, b; von Lützow and Kögel-Knabner, 2010), concluding that it is not responsible for long-term stabilization nor is it the relevant mechanism determining long-term stability of SOM. Therefore, recalcitrance is only relevant during initial phases of litter decomposition (von Lützow et al., 2006). Lützow et al. (2008) identified the key stabilization mechanisms that lead to the formation of different functional SOM pools. The active pool with fresh plant and animal residues is formed by the selective preservation of recalcitrant compounds (turnover <10 years). Biogenic aggregation preserves SOM in the intermediate pool (turnover 10-100 years). A long-term SOM stabilization is controlled by interactions with the mineral soil matrix mainly by microbial processed SOM (Kögel-Knabner et al., 2008; Cotrufo et al., 2013) and by spatial inaccessibility (e.g. microaggregation and formation of hydrophobic surfaces; von Lützow et al., 2008). The formation of passive pools (turnover >100 years) is regarded as hierarchically structured co-action of various processes that are active under specific pedogenetic conditions.

The complex soil N cycle is characterized by diverse N transformation processes and fluxes including both organic and inorganic compounds. There is no direct method to determine turnover rates similar to C research with the ^{14}C radiocarbon dating technique or methods using the isotopic signature in chronosequences of human-induced land use changes (C3 plants to C4 plants with changes in photosynthesis). Our

current understanding of the stabilization processes of N consists of two pathways (Knicker, 2004): (1) An encapsulation into refractory biopolymers or entrapment within stabilized organic residues besides (2) an association of originally labile N with minerals in organo-mineral associations. Overall, an enrichment of N is observed with decreasing particle size, resulting in very narrow C/N ratios in the fine organo-mineral fractions (Stemmer et al., 1998; Kölbl et al., 2006). Sollins et al. (2006) explain this phenomenon with the “onion” model soil that is based on the preferential accumulation of carboxyl and cationic amino compounds directly to mineral surfaces by ligand and cation exchange. This stable inner organic layer then provides relatively nonpolar surfaces for adsorption of a further outer layer onto which other organics could adsorb more readily than onto the unconditioned mineral surfaces (Kleber et al., 2007). The decrease in the C/N ratio confirms the importance of N for the stabilization of SOM via mineral adsorption (Knicker, 2011).

2.2 Fractionation as a tool to identify stabilization pathways of soil organic matter

Physical fractionation allows the separation of SOM fractions that are associated with particles of a specific size, structure, and function ascribed to diverse particular stabilization mechanisms. It is a useful pretreatment for further differentiation of functional fractions (Kögel-Knabner et al., 2008), thought to be stabilized by different mechanisms. The idea of this method is based on the concept that the organization of soil particles plays a decisive role in SOM dynamics. These quantifiable SOM fractions are an approximation to characterize functional SOM pools that are assigned different roles in SOM turnover regarding rate and stability (Christensen, 2001; von Lützow et al., 2007; Sollins et al., 2009). The advantage in comparison to chemical fractionation techniques is the direct relation of the obtained fractions to structure and function of SOM in situ (Christensen, 1992; Cambardella and Elliott, 1994). However, most fractionation techniques do not yield homogenous functional SOM pools (von Lützow et al., 2007). Christensen (1992) provided a comprehensive review of soil physical fractionation techniques, involving both size separation and density fractionation.

Density fractionation as a specific physical fractionation technique isolates soil material by floatation or sedimentation in a heavy liquid (~1.6-2.2 g ml^{-1}) according to particle density. It is used to isolate the light fraction (LF, density below 1.8 to 2.0 g cm^{-3}), which is not firmly associated with soil minerals, from the heavy fraction (Christensen, 1992). This technique was originally developed as a tool to separate primary and clay minerals (Halma, 1969). Since then, it has been refined for SOM research to physically separate SOM into discrete fractions thought to have differing stability (Crow et al., 2007). The LF is considered to have a faster turnover and represents the labile organic matter fraction. The free light fraction (fLF) is mainly related to primary litter input, whereas in soil aggregates occluded light fraction (oLF) is more stable due to occlusion, which is mostly affected by the aggregate turnover of the particular soils (Six et al., 1999a; von Lützow et al., 2007). The heavy fraction comprises organo-mineral associations in which SOM is more processed than in the LF and contains less decomposed plant and animal residues (Christensen, 1992). In contrast to the LFs, these mineral associated fractions are considered to be the more stable fractions containing more processed SOM characterized by a protection of SOM against decomposition due to organo-mineral interactions (von Lützow et al., 2007).

Physical fractionation of soils into different SOM fractions of distinct characteristic turnover times and stability is often applied to investigate soil C pools (Pulleman et al., 2005; Schöning and Kögel-Knabner, 2006; Wagai et al., 2008; Mueller and Kögel-Knabner, 2009), but only a few studies have focused on the stabilization of N through organo-mineral interaction, and these were mainly performed with agricultural soils (Gerzabek et al., 2001; Kölbl et al., 2006; Kong et al., 2007; Kader et al., 2010). The separation of SOM into different functional fractions enables the consideration of key pathways for N stabilization processes, such as chemical recalcitrance, spatial inaccessibility, and the mineral association of SOM (von Lützow et al., 2006). Furthermore, combining soil fractionation and labeling techniques in SOM studies (Kölbl et al., 2006; Mueller et al., 2009), enables a straight and prompt analysis of the decomposition and SOM partitioning processes in the soil. Linking fractionation methods (e.g. particle-size separation) with an incubation approach provides the

advantage of describing SOM stability within discrete fractions obtained from a complex matrix (Crow et al., 2007).

2.3 Climate effects on soil N dynamics

N in soils occurs predominantly in organic forms, mainly as proteins, peptides and amino acids (Schulten and Schnitzer, 1998; Knicker, 2004; Nannipieri and Eldor, 2009) and only a small amount of N is present as inorganic N (N_{min}) (Kelley and Stevenson, 1996). The partitioning of organic N (ON) and N_{min} in soils depends on several biotic and abiotic factors such as soil texture, water availability, quality and quantity of SOM input and microbial N turnover (Tietema et al., 1992; Booth et al., 2005; Nannipieri and Eldor, 2009). Despite great efforts, it still remains challenging to research on many aspects of the N cycle such as N loss via denitrification, competition for N between plants and microbes, and SOM decomposition and N release (Dijkstra, 2009). An enhanced consideration of these aspects of the N cycle is essential to improve our ability to predict how soil N dynamics in terrestrial ecosystems will respond to climate change conditions such as longer drought periods and increasing temperatures. Principal questions still persist when attempting to understand the response of SOM to temperature, i.e. its temperature sensitivity. The present knowledge about temperature sensitivity of SOM mineralization as reviewed by von Lützow and Kögel-Knabner (2009) is determined by several factors: (1) the stability of SOM, (2) the substrate availability, balanced by input, stabilization and mineralization of SOM, (3) the metabolic activity (maintenance – growth) of the microflora and its temperature optima and (4) pedogenetic parameters like pH, oxygen supply, soil water and nutrient availability. There are some indications that when available substrates are exhausted and no other external limitations are restricting decomposition, microorganisms can enzymatically adapt to decompose stable SOM, which has a potentially higher temperature sensitivity than labile SOM (von Lützow and Kögel-Knabner, 2009).

Research on the effects of drought and higher temperatures on soil processes in forest soils has concentrated primarily on soil C cycling, mainly by quantifying soil respiration (Davidson et al., 1998; Luo et al., 2001). Borken et al. (2006) revealed that prolonged

drought periods reduced soil respiration in the organic layer of a forest soil and hence, may cause increases in the storage of soil OC. In a study of Beier et al. (2008) the responses of C and N cycles to global warming were tested, identifying a different behavior: While the C cycling is more sensitive to temperature, the N mineralization was mainly affected by the soil water content. Drought impacts on N_{min} immobilization were also identified by Compton and Boone (2002), indicating that microbial uptake of N_{min} was inhibited by low soil water content. Yet, considerable experimental datasets of impacts affecting N stabilization processes due to summer droughts induced by climate change are still rare. A reason for this gap in science may be the fact that N cycling is based on a multiplicity of N transformation processes and fluxes containing both organic and inorganic N species, interceded by microorganisms and plants. Therefore, the investigation of N cycling processes warrants an extensive consideration of diverse N pools. A common tool to investigate, quantify and characterize soil N pools of different structure and function is the application of physical soil fractionation. An additional approach to investigate the separated fractions regarding their turnover and stability is a decomposing experiment by incubation.

3 Objectives

To investigate and establish a profound insight into N stabilization pathways and turnover processes, a leaf litter decomposition experiment was set up. The major aim was to trace and quantify the stabilization of N released from leaf litter decomposition in different SOM fractions over a period of three years. Another objective was to identify how much label was incorporated and stored in the mineral soil (Figure 1).

Prolonged summer droughts are expected to follow climate change in Central Europe and will lead to reduced soil water availability. Thus, alterations in rates of soil processes as N partitioning among SOM fractions and stabilization are expected. The experiment of **Study II** was established to research, whether drought influences N partitioning between soil, microbial biomass and plants. With this experiment, the

influence of climate change–induced drought on (1) the partitioning of N among SOM fractions and (2) N stabilization was studied (Figure 1).

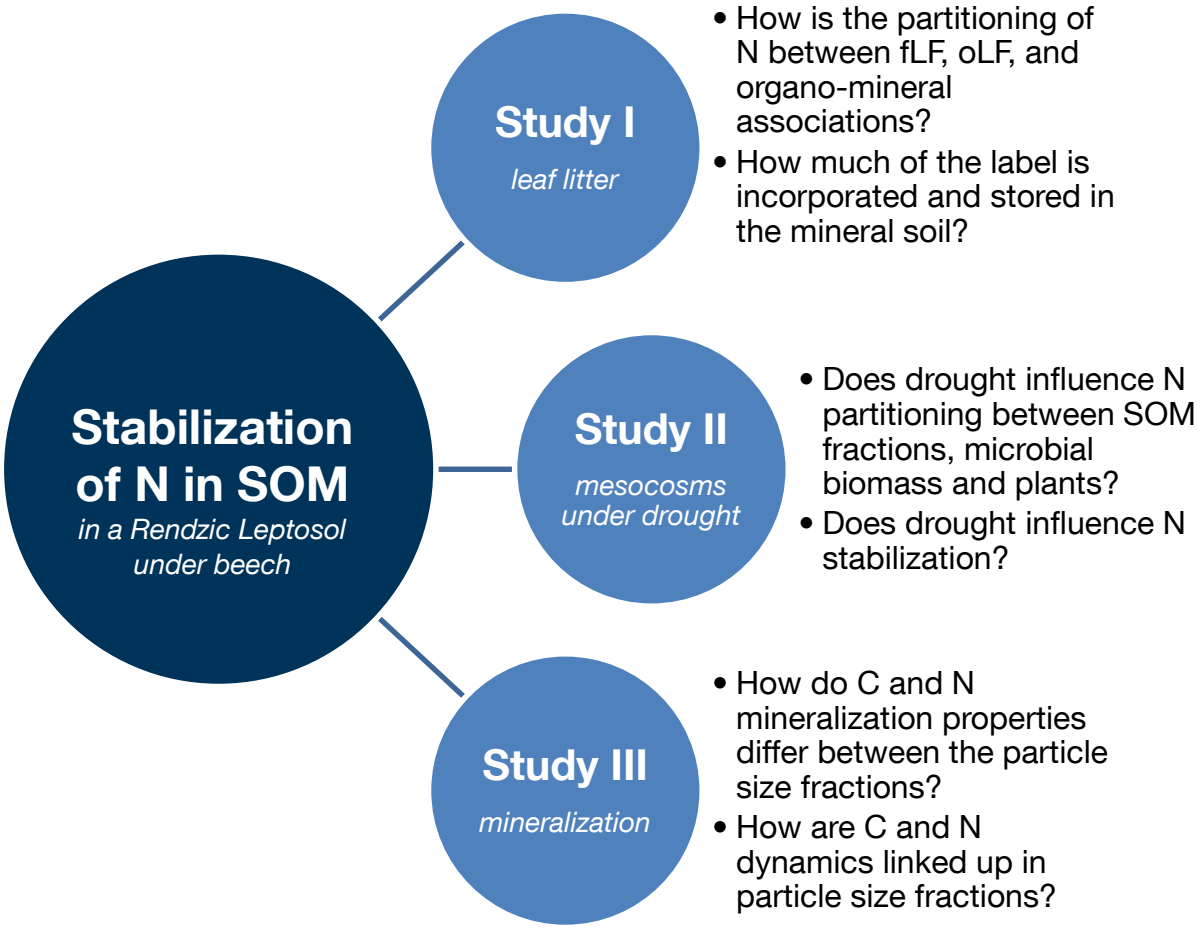

Figure 1: Research questions of Study I-III. SOM: soil organic matter, N: nitrogen, fLF: free light fraction, oLF: occluded light fraction.

The scope of **Study III** was to achieve a more complete understanding of the control of C and N turnover with respect to the different SOM pools and the links between the dynamics. The purpose was to improve the understanding of how C and N are linked up in soils (Figure 1).

In particular, the following specific hypotheses were considered:

H I Clay fractions play a dominant role in the stabilization of N in a Rendzic Leptosol under beech; occluded organic matter is poorly protected within the aggregates which

are turned over rapidly at the calcareous mull humus site owing to the rapid decomposition process and mixing of organic matter and the mineral soil material by bioturbation (**Study I** and **II**).

H II The generally high litter decomposition rates in the Rendzic Leptosol are associated with high proportions of N release and incorporation in the microbial biomass, which in turn favors the stabilization of N in the form of microbial residues in organo-mineral associations (**Study I**).

H III Decomposition is associated with high mineralization immobilization turnover (MIT), which in turn favors the stabilization of ON in the form of microbial residues in organo-mineral associations. The MIT will be influenced by drought conditions (**Study II**).

H IV Less N will be stabilized in organo-mineral associations under drought conditions, due to a lower microbial biomass level (**Study II**).

H V Soil fractions show a different C and N mineralization behavior: SOM is more stable in the clay fraction than in the sand fraction (**Study III**)

The detailed methods and results of **Study I-III** are presented in the original communications attached in the appendix, and therefore, they have only been briefly summarized here.

4 Material and Methods

4.1 Site characteristics

All experiments were conducted at or with material from the Tuttlingen Research Station (47°59'N, 8°45'E) in the Swabian Jura, a low mountain range in southwestern Germany, at an elevation of approximately 780-800 m a.s.l. (Figure 2). The field site has steep slopes with an inclination of 25°. The study site is exposed to a rather low atmospheric N deposition of less than 10 kg N ha^{-1} $year^{-1}$ (Dannenmann et al., 2008).

The area has a cool-moist climate with a mean annual air temperature of 6.5 °C and a precipitation of 854 mm per year (1961–1990) (Dannenmann et al., 2009).

Figure 2: Study site near Tuttlingen in southwestern Germany.
Modified from free image File:Germany_topo.jpg at Wikimedia Commons.

At the field site near Tuttlingen (Figure 3), two model ecosystems are located on different slopes of a valley (Bimüller et al., 2014), thus, showing a cool-moist (NE aspect) and warm-dry local climate (SW aspect). The situation on the SW aspect is assumed to be representative for future climatic conditions (Geßler et al., 2004). Both sites with opposing exposures have a distance of less than 1 km.

European beech (*Fagus sylvatica* L.) is the main species on both slopes, accounting for more than 90% of all the approximately 90-year-old adult trees. Trees show similar population genetics at both sites with a natural selection and potential adaptation account for genetic changes of the adult beech trees on the warm-dry SW slope (Bilela et al., 2012).

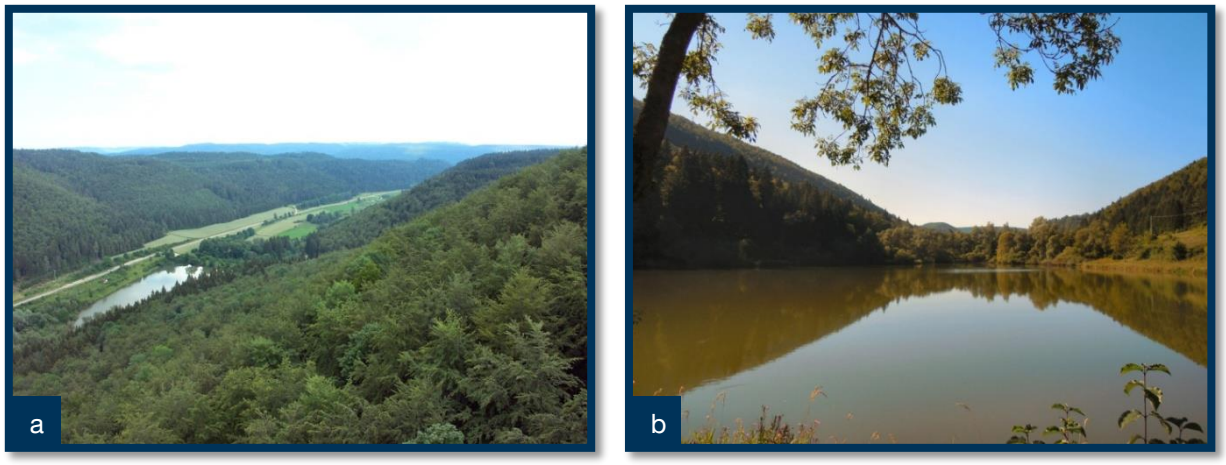

Figure 3: Study area; (a) overview from southwestern slope to the southwestern exposure with the hills of the Swabian Jura in the background (photo by Rainer Gasche, modified with permission); (b) situation at the site: northwestern slope on the left and southwestern slope on the right.

4.2 Physical and chemical soil characteristics

The field experiments were conducted on a Rendzic Leptosol (Skeletic) with Ah-C profile (International Union of Soil Sciences Working Group WRB, 2007) and humus form mull (Figure 4).

Figure 4: Soil profile of a Rendzic Leptosol located in Tuttlingen

Soils with thin profiles developed on weathered bedrock of straight bedded Jurassic limestone and marls, enclosing approximately 45% gravel and stones (Figure 5).

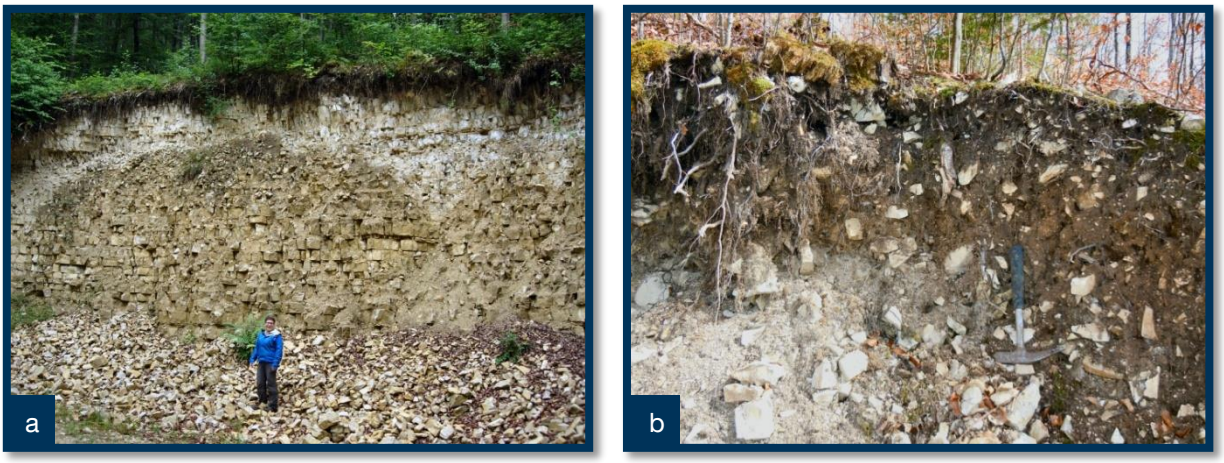

Figure 5: (a) Geological outcrop of Jurassic limestone and (b) shallow soil profiles

The clay content of the soil is high (Table 1). Bulk density is 0.8 g cm^{-3} (Bimüller et al., 2013). The soil is characterized by high bioturbation through the activity of earthworms and also voles. This macro faunal activity including ingestion and mixing of soil results in a high amount of organo-mineral aggregates. The mineralogy of the coarser grain sizes is controlled by quartz; the clay fraction is composed of phyllosilicates, esp. irregular mixed-layer illite–smectite and kaolinite.

Table 1: Soil characteristics of the Rendzic Leptosol (Skeletic) at the study site; OC: organic carbon, IC: inorganic carbon, N: nitrogen; adapted from Elsevier with permission: Soil Biology and Biochemistry 68, Bimüller, C., Dannenmann, M., Tejedor, J., von Lützow, M., Buegger, F., Meier, R., Haug, S., Schroll, R., Kögel-Knabner, I., Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions, 241–251, Copyright Elsevier (2014).

Texture			OC ($g\ kg^{-1}$)	IC ($g\ kg^{-1}$)	N ($g\ kg^{-1}$)	C/N	pH (0.01M $CaCl_2$)
Sand (%) (2000-63 µm)	Silt (%) (63-2 µm)	Clay (%) (<2 µm)	60.7 ± 1.5	0.5 ± 0.2	4.7 ± 0.1	13	6.0 ± 0.2
3.7 ± 0.6	28.5 ± 1.7	67.7 ± 2.2					

4.3 Experimental design of Study I: Leaf litter experiment

^{15}N-labeled beech leaf litter (0.80520 ^{15}N atom% excess) was evenly deposited on the bare soil surface of three selected 2 m × 2 m plots (500 g dry mass litter m^{-2}) at the field

site near Tuttlingen after removing and replacing the original fresh non-decomposed litter in April 2008. To avoid down slope surface transport of litter and deflation, the surface of the plots was covered with a nylon mesh (1.5-cm mesh size), fixed with poles (Figure 6).

Figure 6: Leaf litter experimental setup over three years from (a) April 2008 and (b) May 2008 till (c) September 2010; Photos by Chanjuan Guo (a), Pascale Sarah Naumann (b) and Carolin Bimüller (c). All pictures are modified and reproduced with permission.

The ^{15}N enriched litter was produced by spraying ^{15}N-labeled urea on the foliage of a 10-year-old beech stand once in August of three consecutive years at Puvenelle near Pont-à-Mousson, France (Zeller et al., 1998). Senescent leaves were harvested from the labeled trees in November of a subsequent year, dried at 30 °C, and stored until they were applied as leaf litter in this study.

Litter and soil (Ah horizon, 0–10 cm) were regularly sampled every year after litter application and analyzed for ^{15}N. Subsequently, air-dried soil samples were sieved < 2 mm and fractionated to investigate the partitioning of N via ^{15}N enrichment in different functional SOM fractions.

4.4 Experimental design of Study II: Plant–soil–microbe mesocosm under drought

The experimental approach consisted of a combined space-for-time climate change experiment using intact plant-soil-microbe-mesocosms of a Rendzic Leptosol and an assessment of N stabilization and turnover via a labeling approach with ^{15}N-labeled ammonium nitrate. The relocation of intact plant–soil–microbe mesocosms with a young beech tree from a slope with northwestern (NW) exposure (characterized by a cool-moist microclimate, presenting current climatic conditions) across a narrow valley

to a southwestern (SW) aspect (serving as a warmer and drier model climate for future conditions) provided the experimental basis (Figure 7).

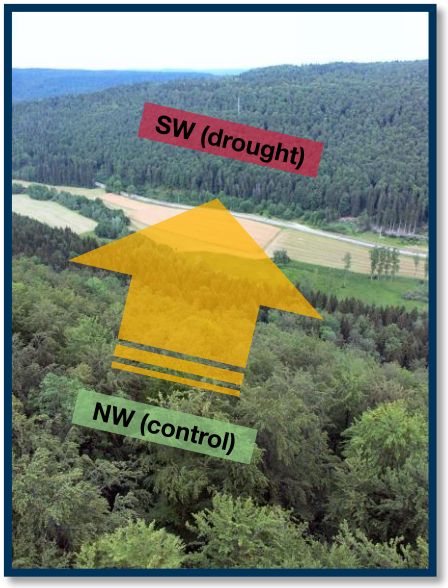

Figure 7: View from northwestern slope to southwestern exposure.
Photo by Rainer Gasche, modified with permission.

The diameter of the soil mesocosms was 162 mm and the length was 150 mm, representing the main rooting zone of beech natural regeneration. Transfers within the area of origin on the NW slope served as in situ controls. After an equilibration period of one year, a homogenous high pulse ^{15}N tracer using $^{15}NH_4{}^{15}NO_3$ at 99 atom% ^{15}N enrichment was applied. Sixteen single volumes of 3 ml solution each were injected into the soil-mesocosms to a depth of 1 and 3 cm each, and another eight injections of 3 ml each to a depth of 6 cm (Figure 8). Ten milliliters of label solution were homogenously spread on top of the soil surface in addition. Hence, the total volume of added label solution was 130 ml, applied to each beech-soil-mesocosm, which contained on average 2.3 kg dry soil.

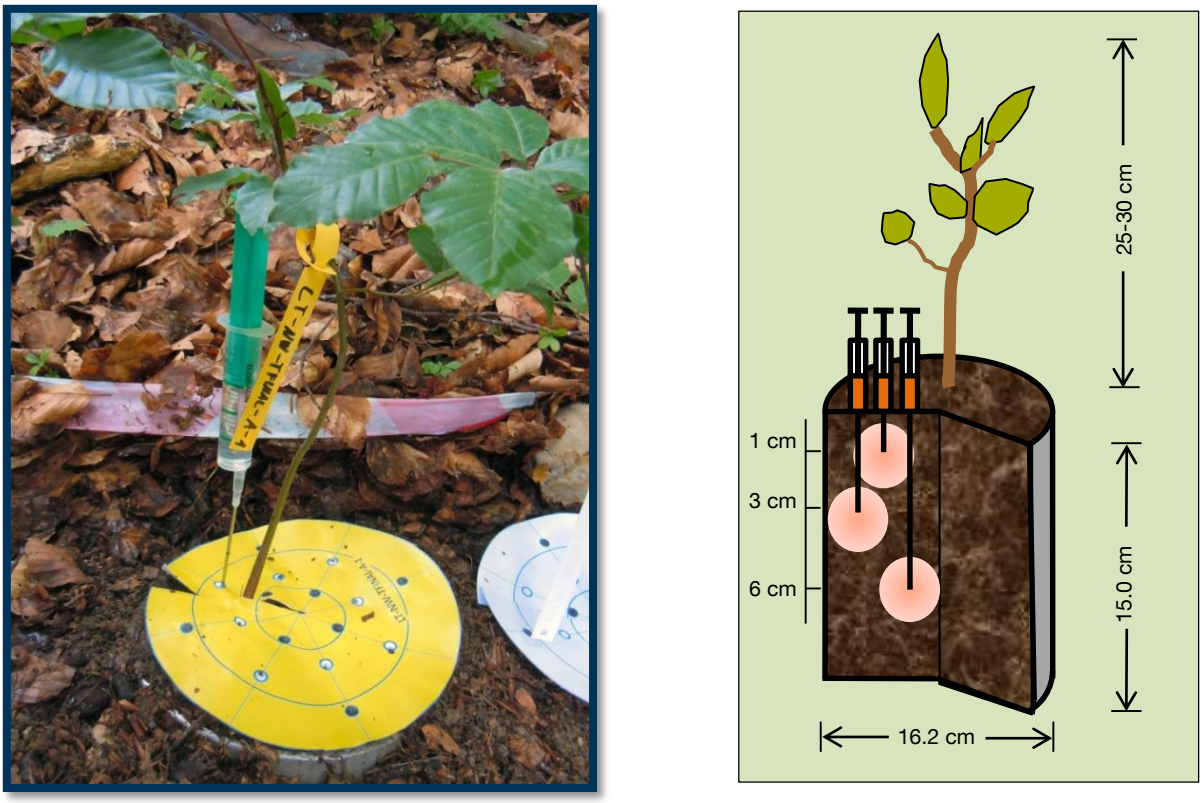

Figure 8: (a) Homogenous and reproducible labeling of the intact plant-soil-microbe-mesocosms (40 injections per beech-soil-mesocosm) with $^{15}NH_4{}^{15}NO_3$ at 99 atom% ^{15}N enrichment using a template, (b) schematic labeling overview

The exposure-induced simulated summer drought conditions were guaranteed, accelerated and intensified through a temporary translucent rain sheltering roof (Figure 9), which was set up for six weeks.

Figure 9: (a+b) Temporary translucent rain sheltering roof constructed over mesocosms on southwestern slope; (c) trenches avoided slope water

Physical fractionation methods (see section 4.6), which were already adapted to the soil from the Tuttlingen site (see **Study I**), and chemical soil extraction protocols were applied to isolate the soil components.

4.5 Experimental design of Study III: Incubation approach studying C and N mineralization of particle size fractions

The controlled laboratory mineralization experiment used an incubation approach of soil fractions to monitor C and N mineralization dynamics, assessing C respiration as well as N mineralization and microbial biomass C and N contents coincidentally. The determination of the specific decomposition rates allowed a judgment of the contribution of the different fractions under specific conditions. Topsoil material from the Tuttlingen site was fractionated into three particle size classes (Figure 10): sand (2000 to 20 µm), silt (20 to 2 µm), and clay (< 2 µm).

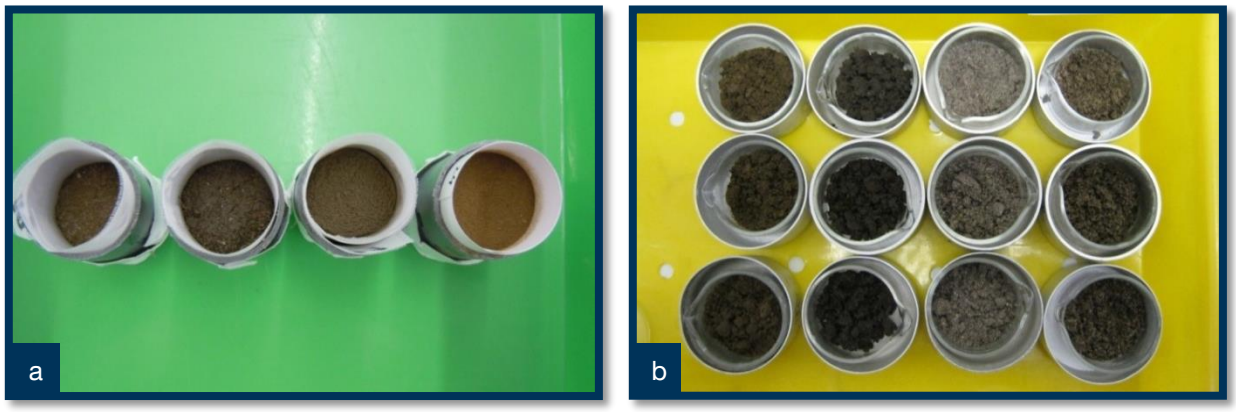

Figure 10: (a) Dry incubated substrates (mixed with quartz sand) before incubation, from left to right: bulk soil – sand – silt – clay; (b) wet substrates (mixed with quartz sand) after incubation in triplicates, from left to right: bulk soil – sand – silt – clay.

Fractionation was adapted to a pure particle-size separation including ultrasonic disruption of soil aggregates, to circumvent repressive impacts on microbial activity by dense liquids (Magid et al., 1996). This method was very sophisticated, because a minimum of 15 g per fraction was needed for every replicate to consider all parameters after sampling of incubated material. Prior to incubation, the fractions were mixed with quartz sand to avoid anaerobic conditions. A replication of n=3 per sampling was selected at seven time points by periodic destructive sampling campaigns during 40 weeks (= 280 d) of incubation. Each vessel with a substrate sample was incubated in an

airtight 0.9 L jar (Figure 11) and stored in a dark room at a constant temperature. An incubation temperature of 20 °C was chosen, which corresponds to more than one full growing season at natural temperatures, however, considering an evaluation of effects induced by higher future temperature conditions projected by climate change. CO_2-C respiration, NH_3-N emission, N mineralization (N_{min}, sum of ammonium and nitrate), microbial biomass C and N, and total C and N were monitored. Regarding N mineralization, ammonium is the direct product of amino acid decomposition, but N mineralization is measured as ammonium plus nitrate production since ammonium is further oxidized to nitrate (Gregorich et al., 1994). SOM in the incubated fractions was considered by a subsequent density fractionation. The chemical composition of selected samples was qualitatively evaluated by ^{13}C-NMR spectroscopy.

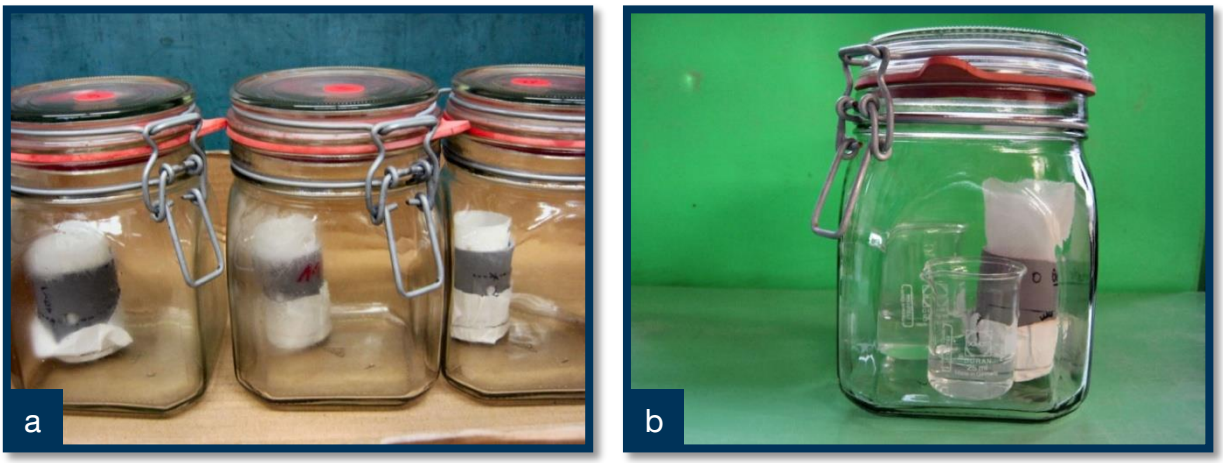

Figure 11: (a) Incubation vessels in airtight jars; (b) incubated replicate for titration, including sodium hydroxide solution and boric acid

4.6 Physical fractionation

A combination of density and particle size fractionation was applied in **Study I** and **II** to differentiate the soil material from the Ah horizon between the LF (mainly particulate organic matter; POM) and the heavy fraction (mineral associated SOM, MIN). For **Study I**, **II** and **III**, a dense solution was produced from sodium polytungstate SPT2 granule by Tungsten (TC Tungsten Compounds, Grub am Forst, Germany). The LF can be separated into the non-protected fLF and the intra-aggregate oLF in soil aggregates (Golchin et al., 1994). The floating fLF (lighter than 1.7 g cm^{-3}), which is mostly related to primary litter input, was extracted by sucking via a water jet pump. To disrupt soil

aggregates and liberate the occluded organic matter, the remaining residual was dispersed ultrasonically with an energy input of 450 J ml^{-1}. Subsequently, the oLF was separated from the mineral residue by a second density fractionation. The heavy fractions, comprising the organo-mineral fractions, were further separated by sieving and sedimentation processes. The separation via sedimentation is based on Stoke's law that allows the calculation of the effective spherical diameter of a settling particle (Cambardella and Elliott, 1994).

Table 2: Overview of fractionation methods used in Study I-III; fLF: free light fraction, oLF: occluded light fraction, LF: light fraction; MIN: mineral associated SOM = heavy fraction.

Study I	Study II	Study III
Combined density and particle size fractionation separating	*Combined density and particle size fractionation separating*	*Particle size fractionation separating*
fLF	fLF	Sand (2000–20 µm)
oLF < 20 µm	oLF < 20 µm	Silt (20–2 µm)
oLF > 20 µm	oLF > 20 µm	Clay (< 2 µm)
Sand (2000–20 µm)	Sand (2000–20 µm)	*Density fractionation of particle size classes after incubation*
Silt (2–20 µm)	Silt (20–2 µm)	LF
Coarse clay (0.2–2 µm)	Clay (< 2 µm)	MIN
Fine clay (< 0.2 µm)		

The applied fractionation procedure was specifically tested in preliminary trials and adapted to this clay-rich soil type in order to achieve an optimized separation of the LF at a density of 1.7 g cm^{-3} by an aggregate disruption with an energy input of 450 J ml^{-1}. This fractionation protocol provided the basis for the fractionation design of all three studies. Slight modifications (e.g. separation of the clay fraction into fine and coarse clay) of the fractionation procedure were used to meet specific needs of the different studies (see Table 2). Therefore, the fine clay fraction was additionally separated from the coarse clay in **Study I**. The incubation experiment in **Study III** was based on a pure

particle size separation prior to incubation. The incubated substrates of this mineralization experiment were subsequently density fractionated.

5 General discussion

5.1 Fate of ^{15}N in a leaf litter labeling experiment in a beech forest

The ^{15}N enrichment of the beech leaf litter decreased very quickly at the beginning, but slowed down and proceeded releasing initial N continuously over time. The litter-released N accumulated mainly on the soil surface, progressively increasing the topsoil's ^{15}N signature. However, the transfer rates to the bulk soil decreased with time since less labeled litter material was available, but even after 876 d, an incorporation of labeled litter material was still observed. Concerning the incorporation of the label after 876 d, 7.1% of the ^{15}N tracer applied with the labeled beech litter was found in SOM fractions (≈ bulk soil) of 0-5 cm depth and another 2% were in the bulk soil of the horizon below in the range of 5-10 cm depth (Figure 12).

Two highlights were observed during the course of the experiment:

First, a quick transfer of ^{15}N-enriched compounds from litter into the mass dominating clay fractions was detected (Figure 12). The processes responsible for this rapid flush of ^{15}N enrichment into the mineral fractions during the early stage of decomposition were leaching of $DO^{15}N$ and NO_3^--^{15}N and hence mass flow and diffusion effects, which led to a rapid attachment and efficient retention of organic and inorganic N species to reactive mineral surfaces. The accumulation of ^{15}N label in the clay fractions was mainly due to leaching and sorption of $DO^{15}N$, besides bioturbation and microbially processed litter. Litter decomposition is commonly considered to be accelerated by the activity of soil invertebrates consuming fresh litter and pre-existing surface SOM. Furthermore, microbial attack is enhanced and the organic matter is spatially linked closer to the mineral phase when processed through the guts of invertebrates (Shipitalo and Protz, 1989). According to Scheu and Wolters (1991), the stabilization of organic matter in the mineral soil horizon due to its binding to clay minerals caused by the burrowing activity of earthworms is a key process in the formation of carbon-rich mull soil of beech

forests on limestone, as investigated here. To some extent, these natural activities are comparable to ploughing or artificial mixing activities of other studies (Aita et al., 1997; Kölbl et al., 2006). These physical intermixing processes may have been accelerated and enhanced by chemical transformations and biotic aggregation of soil biota in **Study I**. The clay's sorption character and the inaccessibility protected the litter-derived N from microbial activity, which subsequently led to a stabilization process.

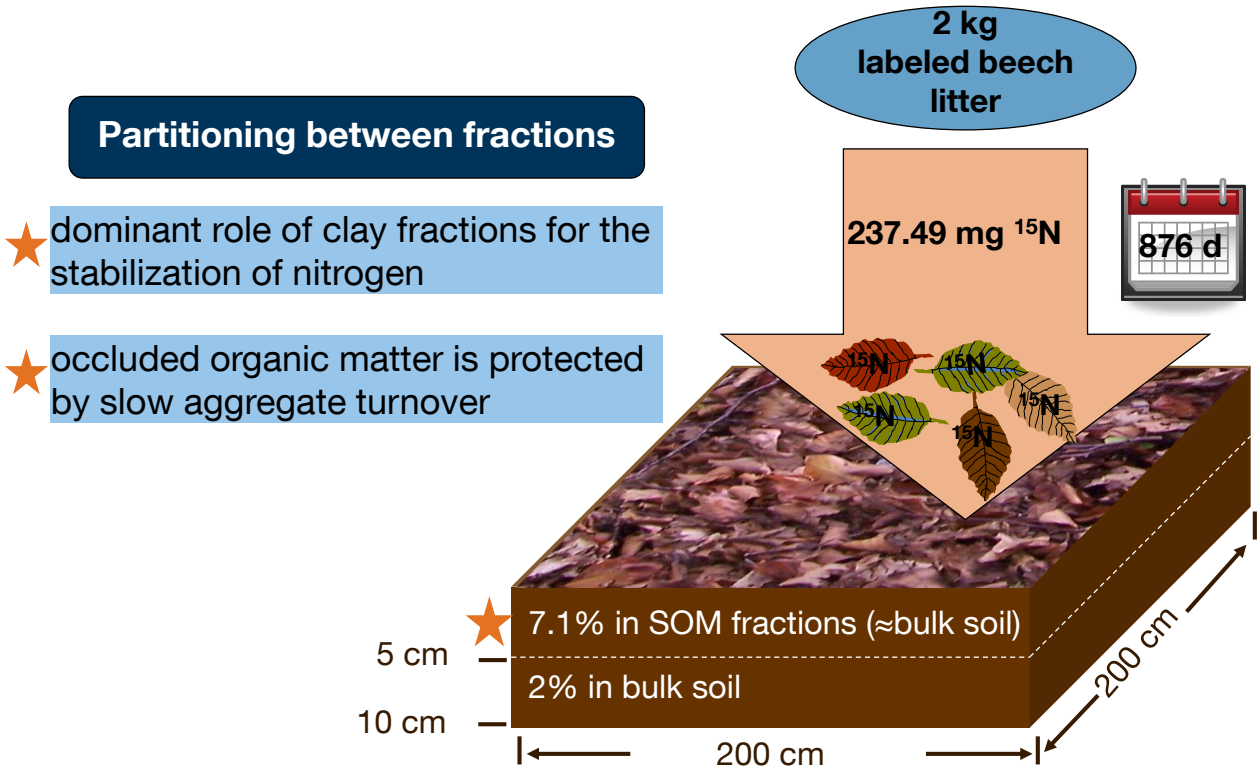

Figure 12: Graphical abstract of Study I summarizing the experimental design and the main findings concerning the partitioning between soil organic matter (SOM) fractions.

Second, the fLF showed higher ^{15}N enrichments than the oLFs, because the oLFs were physically protected due to spatial inaccessibility (Figure 12). Therefore, the input to this fraction was delayed and generally decelerated, displaying a slow aggregate turnover. The late increase in ^{15}N concentration in the oLF < 20 µm was justified by a long process of strong comminution and consequent integration into the aggregate. Unlike Aita et al. (1997) and Kölbl et al.(2006), who incorporated labeled crop residues into agricultural soils, **Study I** did not observe ^{15}N passing through the LFs and a decline in their enrichments at the end of the experiment in the investigated beech forest. Rather,

the 3-year dataset revealed a continuous ^{15}N flux into different functional SOM fractions from decaying litter material.

From these results, the hypothesis **H I** concerning the partitioning between the fractions can be confirmed to a certain extent: the clay fractions play an important role in controlling the stabilization of N as a main sink for over 64% of N; occluded organic matter is protected by slow aggregate turnover. Soil micro-aggregates, especially those < 20 µm, are rather stable over long periods, which may explain the steady state of ^{15}N enrichment during the first harvests and the delayed increase of this fraction at 876 d.
The decomposition of the distributed leaf litter layer lasted longer than expected from literature for a mull humus site (Staaf, 1987). This study provided a profound insight into N stabilization and turnover processes of a Rendzic Leptosol. The experiment clearly identified the relevance of two major long-term N stabilization processes via formation of (1) organo-mineral associations and (2) to a minor extent, soil aggregation in the investigated clay-rich forest soil type.

Another related study by Guo et al. (2013) used the same experimental setup to follow the dynamics of leaf litter N mineralization and nutrition of beech seedlings. The findings from this experiment suggest a primary rather minor contribution of litter N to tree nutrition, but proving the microbial biomass to be the initial dominant N sink. This situation changed over time increasing the long-term dominance of the non-extractable soil fractions in stabilization of N, calculated by subtracting the extractable fractions (N_{min} + dissolved organic nitrogen + N in microbial biomass) from the respective values (N_{tot}) determined from the mineral soil (bulk soil). However, the recovery in the extractable soil fractions was constantly more than an order of magnitude higher than in the plant tissues. Even though, the non-extractable fractions embodied definitely the largest N pool with again one order of magnitude greater recovery values (Guo et al., 2013).

These findings verified hypothesis **H II** with respect to a longer lasting decomposition, which decreased very rapidly at the beginning, but kept on delivering N. A decomposition rate of approximately 20% of the N contained in the litter layer was

measured during the leaf litter decomposition experiment (Guo et al., 2013). This result is in line with the measurements by Pena et al. (2013), determined in a litter bag experiment using the same enriched leaf litter material at the same site and Zeller et al. (2000) for the Vosges mountains. Comparable releases from the original litter are reported for the Tuttlingen site by Dannenmann et al. (2007). Hence, the decomposition rates of the added labeled leaf litter are comparable to that of the native beech litter.

5.2 The impact of summer drought on nitrogen processing and stabilization in soil organic matter fractions

Independently from the objective to identify the impact of drier climatic conditions, this experiment shows again the importance of the clay fraction for stabilization of N in the soil investigated here (**H I**). Within the experimental period lasting for one summer, an altered partitioning of recently applied inorganic ^{15}N between different SOM fractions related to summer drought was detected (Figure 13). Drought mitigated N turnover and led to significantly higher ^{15}N concentrations in the LFs and lower stabilization in organo-mineral associations. The findings showed a retarded stabilization rate, leading to an altered N distribution pattern. This effect was probably influenced by an attenuated N turnover under drought conditions decelerating the mineralization immobilization turnover, as microbial biomass activity is strongly reliant on water accessibility. Hence, microorganisms are less active under drought circumstances and all N cycle processes were retarded. These results correspond to the findings by Gschwendtner et al. (2014), reporting a persistent decline of ammonia oxidizing bacteria and a strong decline in soil nitrate concentrations. This is in line with a throughfall exclusion experiment by Schmitt and Glaser (2010) leading microbes to suffer from water stress. They concluded that warmer and drier weather led to a dominance of fungi while a cooler and moister regime favored bacteria.

Prolonged summer droughts of **Study II** modified the stabilization dynamics since (1) water as a transport agent was missing and (2) the induced inactivity or even dormancy of microorganisms may diminish the N transfer to stabilization pathways (**H IV**). A decelerated stabilization of N in organo-mineral associations and a contemporary

prolonged retention in the labile fractions increase the possibility of substantial N losses during heavy precipitation events after consecutive summer droughts. These weather extremes are expected to occur more frequently in Central Europe in the 21st century as predicted by future climate change scenarios (Intergovernmental Panel on Climate Change IPCC, 2013).

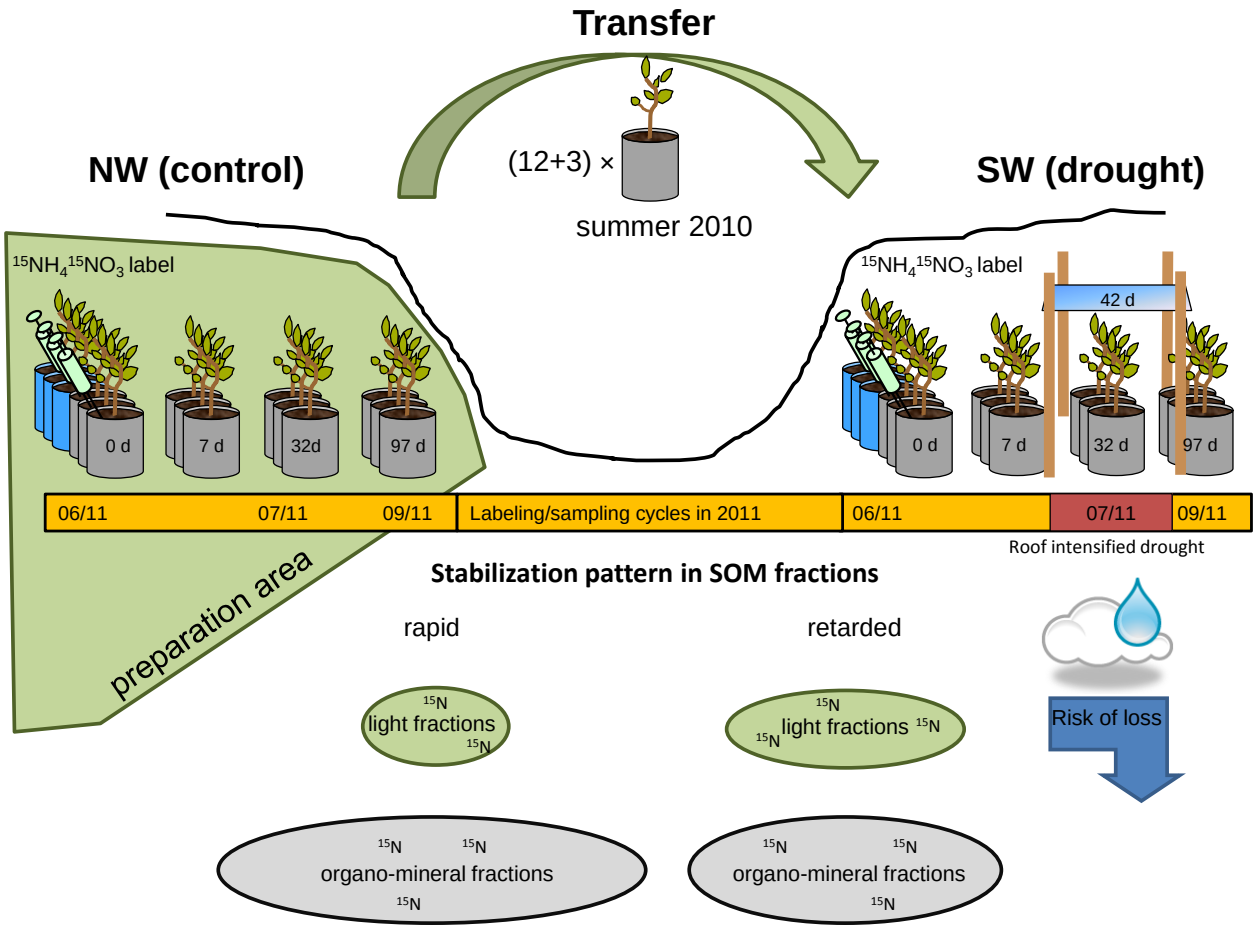

Figure 13: Graphical abstract of Study II illustrating the experimental design including the transfer of mesocosms from the northwestern (NW) to the southwestern (SW) slope and the drought impact on the stabilization pattern in soil organic matter (SOM) fractions. Reprinted by permission from Elsevier: Soil Biology and Biochemistry 68, Bimüller, C., Dannenmann, M., Tejedor, J., von Lützow, M., Buegger, F., Meier, R., Haug, S., Schroll, R., Kögel-Knabner, I., Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions, 241–251, Copyright Elsevier (2014).

Evans and Burke (2013) detected a similar accumulation of N under drought that is vulnerable e.g. to gaseous losses. The experiment of **Study I**, showing a modified tracer partitioning in different SOM fractions induced by summer drought, may serve as a central factor in modeling prospective reactions of beech forest ecosystems.

The above-described **Study II** was a satellite investigation embedded into a comprehensive interdisciplinary ecosystem project researching N turnover in European beech forests under climate change conditions by the Beech Research Group. This experiment was designed to answer the question, whether bioavailable N in the soil declines as a consequence of longer drought periods resulting in enhanced competition between soil microorganisms and beech seedlings. The physiological mechanisms underlying the drought sensitivity of beech are not yet well understood. Considering soil N biogeochemistry, sequestration into SOM, soil microbiology, mycorrhiza ecology and plant physiology in a unique experimental approach in situ, a close relationship between soil water availability, soil microbial N turnover and N nutrition of beech was identified. This experiment explains how reduced soil water in a changing climate results in unexpectedly strong nutritional limitation of beech due to drastically reduced nitrification and soil nitrate availability. Nitrate was the major N source for beech natural regeneration. Limited soil water content caused a persistent decline of ammonia oxidizing bacteria and hence, a massive decrease in gross nitrification and nitrate availability in the soil. Thus, nitrate and total N uptake by beech seedlings were strongly reduced. Consequently, impaired microbial provision of bioavailable N may be a stressor for growth of beech seedlings in addition to plant physiological limitations under reduced soil water availability, enhancing the vulnerability of European beech forests under predicted climatic stresses. The nutritional deficiency will be additionally exacerbated by enhanced N leaching after prolonged drought due to retarded stabilization of microbial N in organo-mineral associations as presented by **Study II**. Using climate predictor-driven statistical distribution modelling, a decline by 78% of potential beech distribution on calcareous soils in Europe by the year 2080 was computed. Therefore, the present results question the sustainability of the widespread beech ecosystem on marginal soils already in the near future. This comprehensive experiment generated an important data basis for the further development of biogeochemical models that will be used to model the N turnover. By highlighting the relationships between water stress, N cycling and beech N nutrition, the results point to developing mitigation alternatives to increase forest resilience and adaptation potential

in a changing climate. Nutritional restrictions could be counteracted by fertilization to rise levels of bioavailable N in soil. Another opportunity is silvicultural reduction of stand density or conversion via drought-robust, deep-rooting trees. These options can increase the beech forest stands' resilience to climatic changes.

5.3 Availability of carbon and nitrogen in soil particle size fractions

Respiration rates declined over time following the order sand > clay > silt. The fractions respired between 10.4% (sand fraction), 8.8% (clay fraction) and 4.4% (silt fraction) of total OC. During the fractionation, mobile C and N species, soluble organic C and N, as well as microbial remnants were leached from larger grain sizes to the clay fraction. This experimental design therefore overrated the rapid pool of the clay fraction at the start of the incubation due to these fast mineralizable leachates. At the beginning, OC was mineralized more rapidly in the clay fraction than in the silt or the bulk soil. With the reminder of the experiment, the respiration curve of the clay fraction declined to a level below the C mineralization curve of the bulk soil and was characterized by a basal respiration of the more stable pool.

There is some scientific discussion in literature about the fact that N present in the clay and silt fractions is per se "stabilized" (**H V**). For instance, Dorodnikov et al. (2011) observed constant turnover rates of C in SOM fractions during their experiment, data which neither confirm nor neglect the assumed stabilization of SOM taking place in the soil mineral fraction. Steffens et al. (2009) found a comparably fast SOM turnover in organo-mineral associations indicated by increased radiocarbon concentrations. **Study III** showed that only the labile C species dislocated to the clay fraction during fractionation were not stable and mineralized rapidly. Considering this pretreatment, **Study III** revealed a low basal respiration by the clay fraction (**H V**).

N mineralization followed the order clay > silt > sand. When summing up the mineralization rates of the single fractions, the values for CO_2-C equaled the bulk soil, whereas the mathematical recombination of N_{min} in all fractions was significantly less than in bulk soil (Figure 14). Hence, C mineralization was not affected by the damage of the aggregated soil structure via fractionation in spite of a better accessibility and

exposition of SEOC, whereas fractionation reduced N mineralization. Lower metabolic quotients in the bulk soil indicated a more efficient microbial C mineralization compared to the calculated sum of fractions, but also a better bioavailability of SOM in the single fractions after fractionation. Additional effects were an inefficient C mineralization of the single fractions and a decreased N bioavailability in the clay fraction triggered by the generation of new surface areas adsorbing favorably mineral or organic N compounds (Kleber et al., 2007). All fractions mineralized less N per unit C in relation to the bulk soil.

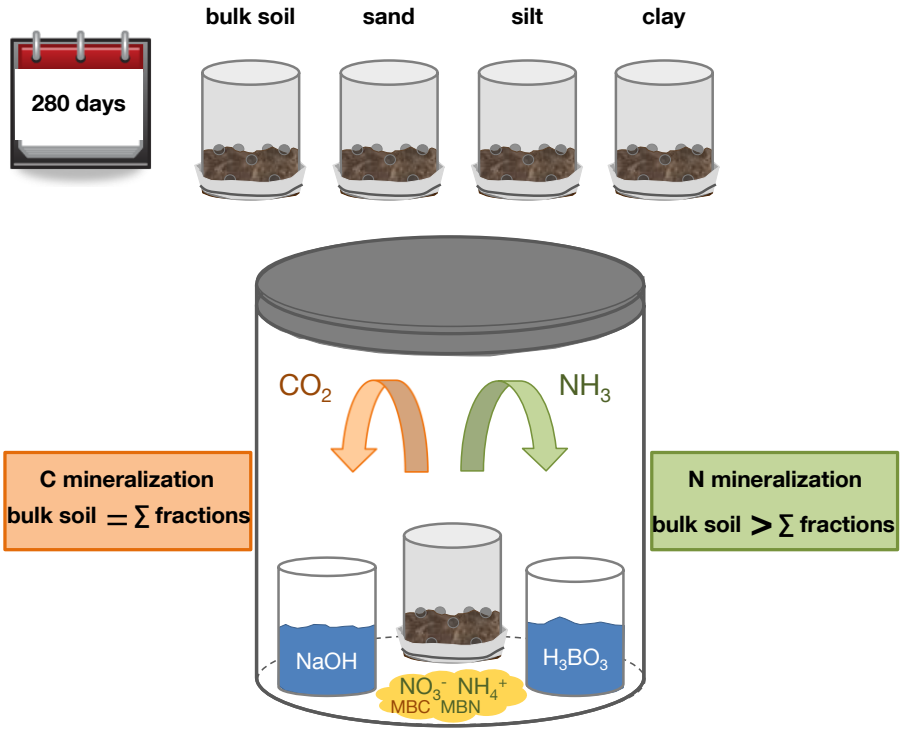

Figure 14: Graphical abstract of Study III introducing the experimental design using a jar as an incubation chamber for carbon (C) and nitrogen (N) mineralization of particle size fractions. The mineralization behavior of sand, silt and clay was compared to the bulk soil. Reprinted by permission from Elsevier: Soil Biology and Biochemistry 78, Bimüller, C., Mueller, C. W., von Lützow, M., Kreyling, O., Kölbl, A., Haug, S., Schloter, M., Kögel-Knabner, I., Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil, 263–273, Copyright Elsevier (2014).

Organic matter in the sand fraction contained mainly particulate organic matter present as light material comprising partly decomposed plant remnants. The quantity of light material in the clay fraction was below detection limit, because almost all SOM was tightly bound to mineral surfaces. O/N-alkyl C indicating low recalcitrance prevailed in the sand and in the clay fraction, but the C/N ratio of organic matter narrowed with

decreasing particle size. C mineralization rates, SEOC contents, and microbial biomass C and N contents decreased strongly in the clay fraction towards the end of the incubation. This indicates a C limitation of the microorganisms in the clay fraction, consequently leading to an inhibition of N immobilization. Along with its availability, SOM must be also accessible for the microbial biomass to guarantee an efficient C and N mineralization. **Study III** showed that C/N and alkyl C to O/N-alkyl C ratios of organic matter are not appropriate indicators for bioavailability describing the biological decomposability in fractions where organic matter is mainly stabilized by spatial inaccessibility and by organo-mineral interactions. These parameters are appropriate indicators for decomposition of plant residues on or also in the mineral soil, but they should not be used for mineral-associated SOM. The specific interactions of both C and N containing components with the mineral matrix strongly control the mineralization dynamics, leading to a decoupling of these processes.

5.4 The role of different physical fractions in SOM stabilization

Study I-III generated quantitative data on the turnover and transfer of N to SOM fractions and thus, the relevance of major long-term stabilization processes via formation of organo-mineral associations. At the same time, the three experiments researching soil from the same site delivered data on soil N cycling on a process-oriented scale and further in situ information on responses of N stabilization processes to climate change conditions. The combination of soil fractionation protocols with labeling techniques allowed a prompt analysis of the partitioning of SOM in functionally different pools with diverse stability (Kölbl et al., 2006; Mueller et al., 2009).

The leaf litter experiment (**Study I**) revealed that the major sink for N released from leaf litter is N in stable, non-extractable SOM fractions stabilized via organo-mineral associations in a clay-rich Rendzic Leptosol. The LFs acted as a transitory pool of the decomposition process between litter and mineral-associated organic matter (Christensen, 2001). The bioturbation impact did not only comprise of the physical dislocation of organic matter from the litter layer into the mineral soil, but involved also litter feeding with a consequent excretion of casts as hotspots for mineralization and

microbial activity (Fahey et al., 2013; Guo et al., 2013). **Study III** confirmed the dominant role of the clay fraction for N storage within the studied soil, but coincidentally showed a function as an obvious N source for mineralization to some extent. Von Lützow et al. (2007) reported a wide range of ^{14}C mean residence times for the clay fraction with differences between the coarse clay and the fine clay through lower mean residence and turnover times in the finer fraction. Laird et al. (2001) ascribed the variance of stabilization in clay subfractions to a change in the mineral composition and a consequential selective sorption of SOM with different functional groups on specific clay minerals.

A literature review of comparable leaf litter experiments revealed diverse results concerning the roles of different fractions in the stabilization process: Zeller et al. (2001) observed a high variability for litter N dynamics among three forest sites, depending strongly on site-specific parameters like soil type (Cambisol and Leptosol) and humus form (moder and mull-moder). Hatton et al. (2012) found only ~40% of litter-derived N in organo-mineral fractions of a Cambisol (14% clay content) after a decomposition period of four years. These results indicate that N stabilization is highly dependent on soil type, texture and N distribution and that these soil specific characteristics have to be considered when investigations of N cycling in a plant soil system are performed. However, Hatton et al. (2012) showed that the formation of organo-mineral associations is a central mechanism for decadal- to longer-term preservation of litter-derived N in forest soils.

Mull humus is commonly associated with high levels of nutrient turnover including a rapid and indirect N and C cycling (Staaf, 1987; Ponge, 2013). This fast decomposition and litter disappearance is supposed to be rapid enough to almost completely remove the litter layer before the next autumn leaf-fall (Bocock et al., 1960; Staaf, 1987). Staaf et al. (1987) reported differences in decomposition velocity between mull sites dependent from their richness of the humus. The decomposition of the applied litter at the clay-rich beech forest site near Tuttlingen presented itself slower than expected

(**H II**), since labeled material was still found on the soil surface in the third year of the experiment.

The activity of soil microorganisms is highly dependent on soil moisture (**HIII**, **Study II**) with a retarding drought impact on the MIT. All N cycling processes in the drought-treated plant-soil-microbe system slowed down because the microorganisms were less active. The MIT was delayed, hence impeding the transfer into the mineral associations. This results in an extended residence time in the labile fraction, where N is less stable than in organo-mineral associations. The risk of leaching losses under extreme rainfall events increases. This might not necessarily be because of a lower microbial biomass level due to partial kill-off reported in literature (**H IV**; Sparling and Ross, 1988; Van Gestel et al., 1993a, b), but also to a pure inactivity of microorganisms as revealed by **Study II**.

The mineralization experiment (**Study III**) presented the different C and N mineralization behavior within particle-size fractions. Common stabilization concepts (Sollins et al., 1996; von Lützow et al., 2006; von Lützow et al., 2008) define the clay fractions dominated by organo-mineral associations to be the most stable pools of SOM. However, some studies (Steffens et al., 2009; Dorodnikov et al., 2011) assume that SOM in organo-mineral associations is not as stable as it is supposed to be. **Study III** displayed that only the labile C species transferred to the clay fraction during fractionation were not stable and mineralized rapidly. Considering this methodological pretreatment, SOM was more stable in the clay fraction than in the sand fraction regarding soil OC (**H V**). Slower SOM turnover rates in the clay fractions compared to the sand fraction resulted from a combination of all three process groups of stabilization including particularly spatial inaccessibility, interactions with minerals and metal ions besides recalcitrant properties (von Lützow et al., 2007).

To summarize, added leaf litter material decomposed more slowly in the field than expected from a mull site. The clay fraction was identified as main N sink. The potentially intermediate mineralization rates under optimized conditions indicate that the N stabilization in clay-sized fraction is lower than might have been expected.

5.5 Advantages of combining field and laboratory experiments

Study I and **II** were conducted as field trials to investigate the specific parameters of N stabilization in SOM fractions in situ in its natural, undisturbed environment. Influencing factors were controlled to an extent that is permitted by the situation at the natural site (e.g. roof establishment in **Study II**). These approaches enabled studying N dynamics by tracing the ^{15}N label as a specific cohort through the SOM fractions. These labeling techniques allowed a detailed study of the N cycling and a thorough evaluation of drought impact on these processes. The analysis of **Study II** is explicitly novel in combining new isotope-based techniques for in situ quantification of N turnover in intact plant-soil-microbe-mesocosms with a space-for-time climate change experiment. The results obtained from the two field experiments have a high ecological validity and can be generalized easily to the "real world".

By contrast, the setting of a laboratory experiment (**Study III**) had the advantage of controlling and optimizing several influencing parameters in parallel to minimizing their effects. The incubation experiment of **Study III** was designed to investigate the potential behavior (Gregorich et al., 1994) of the various fractions under controlled conditions and without the influence of growing plants. This could not be examined in the field, because soil can only be fractionated in a laboratory, breaking up its natural matrix. Temperature for the whole period of 280 d was set to 20 °C and moisture tension was adjusted to field capacity for all incubated samples in **Study III**. Compared to the annual mean temperature, the chosen high temperature accelerated the mineralization. Therefore, microbial activity corresponded to more than one full growing season at natural temperatures. This allowed an evaluation in fast motion. Under field conditions, the amount of C and N available for mineralization in the different fractions may change due to the effects of higher temperature with future climate change (see **Study II**). Microbial N dynamics in the field would be affected e.g. by N uptake of plants (Dijkstra, 2009), loss of dissolved organic and inorganic nitrogen by leaching, and by inputs of labile SOM by rhizodeposition and litter. The microbial N demand would increase in the field while its supply would be reduced at the same time, thus increasing the affinity to immobilization (Weintraub and Schimel, 2003). **Study III** does

not provide an additional delivery of SOM to the incubated fractions. However, it is known from **Study I** that SOM input to the clay fraction is important and comparably fast. This more precise experimental manipulation, its standardization and random allocation of the sample offers not only a repeatable and reproducible investigation, but also a focusing on special properties of SOM fractions.

Therefore, this unique combination of field (**Study I** and **II**) and laboratory (**Study III**) experiments was carried out for this thesis to profit from the advantages of both. This approach delivered a valuable complement, thereby obtaining insights into stabilization and processing of N in SOM benefitting from field experiments which deliver a proximity to the real world and a detailed perspective obtained by the laboratory mineralization experiment.

5.6 Advantages from the leaf litter experiment over litter bag studies

The approach using ^{15}N-labeled leaf litter has hardly ever been applied, because it is expensive and time-consuming to produce labeled organic material (Zeller et al., 1998; Langenbruch et al., 2014). However, it is worth doing, since the long-term fate of N released from litter cannot be simulated by single high-pulse label of isotopically enriched organic or inorganic compounds. N from leaf litter is rather constantly released in the form of a wider variety of chemical compounds and incorporated into soil via several physical processes like leaching and bioturbation (Guo et al., 2013). Moreover, N_{min} additions are frequently applied during the course of other experiments to fulfill an appropriate isotopic signal of soil and plant tissues in subsequent mass spectrometry, thus exceeding nutrition balances.

The experimental setup of defined plots where leaf litter was deposited on the bare soil surface was specifically adapted to the mull humus form. This design was aimed to give access for macro fauna and to allow barrier-free incorporation of the litter material into the mineral soil. Nevertheless, large soil animals may dilute the label signal due to their mobility and their ability also to feed outside the experimental plots (Caner et al., 2004). The exposition of the labeled material on the forest floor permitted further hyphal and root ingrowth (Pena et al., 2013). However, this approach was not able to assess

the losses of litter material that may have occurred due to edge effects of the slope, as a result of colluvial and eluvial transport. A quantification of the mass loss and the remaining leaf litter on the soil surface was not possible with this setup. The chosen arrangement simulated more natural conditions in comparison to classical litter mash bag studies, which also hinder and therefore underestimate mycelia in the soil (Guo et al., 2013).

5.7 Considerations during fractionation

All physical fractionation protocols comprise several steps of soil dispersion, followed by density and/or size separation (Moni et al., 2012). Sonication has been widely applied to disperse soil prior to separation of fractions as one of the first steps during fractionation (Gregorich et al., 1988, 1989; Cambardella and Elliott, 1994; Gregorich et al., 2006). However, four byproducts from fractionation procedure including ultrasonic dispersion must be considered: (1) the method does not destroy all micro-aggregates (Chenu and Plante, 2006; Moni et al., 2012) compared to texture analysis, (2) SOM is released through the break-up of aggregates and further comminuted (Christensen, 2001; Gregorich et al., 2006), increasing the accessibility, (3) microorganisms are killed (Christensen, 2001) due to ultrasound and freeze-drying and (4) SOM and microorganisms are redistributed and leached into the clay fraction (Cambardella and Elliott, 1994; Mueller et al., 2014). When using sedimentation processes in the course of particle size fractionation, Stokes' law must strictly speaking only be applied to spherical shapes of settling particles with a density of 2.65 g cm^{-3} (Moni et al., 2012). Otherwise, the fractionation will result in an impureness of larger particles in smaller fractions (Cambardella and Elliott, 1994). Considering these consequences, fractionation enables a comparison and classification of soil fractions and the bulk soil, which is otherwise not accessible. These byproducts were taken account of in the experimental design of **Study III**, where the fractionated and incubated substrates were inoculated to circumvent the partial kill-off of microorganisms in the isolated fractions. All other effects were considered in the discussion of the results: A comparison of the texture described in **Study I** did not provide the same distribution compared to

fractionation (**Study I-III**). However, the texture analysis used different size classes and chemical dispersing agents.

Density fractionation was performed through floatation and decantation procedures using sodium polytungstate (SPT). It is known to solubilize a certain proportion of C, which may redistribute across fractions or becomes lost with the supernatant (Six et al., 1999; Chenu and Plante, 2006). Studies by Crow et al. (2007), Kramer at al. (2009) and Sollins et al. (2009) investigated respectively refer to contamination effects from N-rich SPT by showing a preferential mobilization and loss of the light isotope. Kramer et al. (2009) ascribe this effect to the presence of ^{15}N-enriched ammonium in high N SPT and caution against N levels exceeding 0.5 mg g^{-1}. The influence of the enrichment via the dense liquid was tested in a preliminary experiment prior to **Study I-III**. It was shown, that SPT2 had an N content of 0.65 mg g^{-1}, and therefore, increased the enrichment in the isotopically heavy N isotope ^{15}N in the investigated sample. However, these problems were circumvented by measuring blanks/controls in terms of the natural abundance which were subtracted from the enriched samples in **Study I** and **II**. Additionally, recycling of SPT was avoided and a freshly prepared dense liquid was used to fractionate each sample. Moreover, previous studies (Magid et al., 1996; Crow et al., 2007) show that SPT has a strong inhibiting effect on microbial activity in subsequent mineralization studies of isolated density fractions. Therefore, this fractionation method for preparation of the incubation substrates was avoided in **Study III** in favor to a pure particle-size separation.

Considering these issues, the main advantage of fractionation methods is the isolation and analytical characterization of individual soil fractions, separated from a complex soil matrix, which are otherwise not accessible.

6 Conclusions

The main focus of this thesis was to research on the partitioning and major stabilization processes for N in SOM. Physical fractionation has been used to identify sensitive fractions and pools of SOM. In combination with ^{15}N in situ labeling approaches, the

experimental designs provided a deeper insight into the N cycle and stabilization mechanisms.

Study I and **II** point a strong sorption capacity of the minerals isolated in clay fractions, mostly phyllosilicates, towards the litter-derived N at the initial stage of the litter decay, respectively recently applied N cohort. In addition to this, the generally high clay content, which distinctly dominates the mass distribution of the investigated soil, is responsible for the stabilization of N (**H I**). Furthermore, a high degree of aggregation that may protect the SOM was found. The sorption character of the organo-mineral fractions in addition to the inaccessibility of aggregates protect SOM respectively litter derived N (**Study I**) from microbial activity, thus resulting in stabilization. The aggregate turnover presented itself more slowly than expected (**H II**), since the plant debris occluded in aggregates occurred only in the third year at the final stage of the leaf litter experiment (**Study I**). This oLF therefore acts as a transitory storage of recently applied N respectively SOM. Incorporation of litter-derived ^{15}N in the mineral soil occurred predominantly in the coarse and fine clay fractions, with a surprisingly rapid transfer of ^{15}N completed within 140 d. ^{15}N in organo-mineral associations accounted for more than 60% of the total ^{15}N recovered, acting as a rapid sink of ^{15}N derived from leaf litter in the long-term. This outcome emphasizes the central role of organo-mineral associations for the stabilization of N in the investigated soil. These stabilization processes will be inhibited by desiccation in terms of climate change induced prolonged summer droughts in Central Europe as deduced from the results of **Study II**. Microbial biomass activity is heavily dependent on water accessibility, with retarded MIT under drought conditions (**H III**). **Study II** showed that recently applied N_{min} responded with higher ^{15}N concentrations in the LFs and less ^{15}N stabilized in organo-mineral associations under drought circumstances (**H IV**). When microorganisms are less active in a drought setting, they slow down all N cycle processes. The prolonged residence time of N in the labile fraction rises the risk of leaching losses with heavy precipitations, which are predicted for Central Europe. The retarded stabilization speed suggested by **Study II** may initiate an altered N distribution pattern.

The information about decomposition and biodegradability of specific SOM compartments obtained by **Study III** allowed an evaluation of SOM stability, associated with these individual fractions. Labile SOM compounds in the clay fraction were recognized as byproducts of the fractionation procedure, thus neither confirming nor neglecting the assumed hypothesis **H V**. The incubation experiment revealed that the C/N ratio as well as the alkyl C to O/N-alkyl C ratio as an indicator for decomposability should not be used for a description of soil fractions that are mainly stabilized by spatial inaccessibility and by organo-mineral interactions. The concrete bioavailability of C and N defines the mineralization pattern in the different SOM fractions. Here, the clay fraction is defined by inhibited N immobilization due to C limitation. In the sand-sized fraction, which encloses a litter-like POM fraction, C is vacant for mineralization, but N is the limiting factor. There is consensus that C and N mineralization are thoroughly linked processes during the decay of plant residues on or in the soil. **Study III** suggests that C and N processes are decoupled in the mineral-associated fractions of the soil, as the interactions of both C and N containing compounds with the mineral matrix strongly control the mineralization dynamics.

Study I shows that litter-derived N becomes progressively and rapidly associated with aggregates and adsorbed onto mineral surfaces. Considering future challenges, this interface between plant debris and mineral soil particles has so far remained uninvestigated. Spectroscopic methods using scanning electron microscopy (SEM) and NanoSIMS (secondary ion mass spectrometry at the nanoscale) may provide a useful approach to study this interface at the nanoscale. An additional further research aspect would be the quantification of the different label losses via leaching in the joint aquifers of limestone karst systems at the field site or gaseous, colluvial and eluvial losses, which are particularly relevant in **Study I**. An additional experiment completing the climate change issue raised in **Study II** could be a soil rewetting experiment of the drought mesocosms and the impact of partitioning and stabilization of N within SOM fractions. This has already been studied for bulk soil in related experiments described in section 5.2. The impact on N stabilization within the various SOM fractions respectively,

and its endangered leaching losses from fLF could be tested using such an experimental approach.

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Appendix

Study I

Bimüller C, Naumann P, Buegger F, Dannenmann F, Zeller B, von Lützow M, Kögel-Knabner I (2013): Rapid transfer of ^{15}N from labeled beech leaf litter to functional soil organic matter fractions in a Rendzic Leptosol. Soil Biology and Biochemistry, 58, 323–331.

Study II

Bimüller C, Dannenmann M, Tejedor J, von Lützow M, Buegger F, Meier R, Haug S, Schroll R, Kögel-Knabner I (2014): Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions. Soil Biology and Biochemistry, 68, 241–251.

Study III

Bimüller C, Mueller C W, von Lützow M, Kreyling O, Kölbl A, Haug, S, Schloter M, Kögel-Knabner I (2014): Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil. Soil Biology and Biochemistry, 78, 263–273.

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by

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Rapid transfer of ^{15}N from labeled beech leaf litter to functional soil organic matter fractions in a Rendzic Leptosol

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ABSTRACT

Our main objective was to trace and to quantify the stabilization of nitrogen released from litter decomposition in different functional soil organic matter fractions. To identify the fate of nitrogen in a free-range experiment, ^{15}N-labeled beech litter was deposited on the bare soil surface of three 2 m × 2 m plots on a Rendzic Leptosol under beech (*Fagus sylvatica* L.) with mull humus form near Tuttlingen (Swabian Jura, Germany). The ^{15}N composition of bulk soil and soil fractions was monitored for three years by sampling the litter layer and the Ah horizon (0–5, 5–10 cm) after 140, 507, and 876 d. A combined density and particle size fractionation procedure allowed the isolation of different functional soil organic matter fractions: free light fraction, occluded organic matter, and organo-mineral associations. The first flush in the ^{15}N enrichment was observed in the bulk soil within 140 d, due to plant debris transferred to the free light fraction by probably bioturbation and soluble compounds being leached from the litter directly to the clay fractions. The observed rates within the first 140 d indicated a quick transfer of ^{15}N-enriched compounds from litter into the free light fraction, with a rate of 0.07 μg kg^{-1} d^{-1}, and to the clay fractions, with a rate of 0.31 μg kg^{-1} d^{-1}. In contrast, transfer to the occluded light fractions was delayed, with rates of 0.01 μg kg^{-1} d^{-1} (> 20 μm) and 0.001 μg kg^{-1} d^{-1} (< 20 μm), respectively. After 876 d, we recovered 9% of the added label in the 0–10 cm soil horizon, of which more than 4% was found in the organo-mineral fraction (0–5 cm), nearly 3% in the light fractions (0–5 cm), and another 2% unspecified in the bulk soil of 5–10 cm depth. We therefore conclude that the clay fractions act as the main sink for the recovered ^{15}N. The rapid incorporation and the high preservation of ^{15}N in the clay fractions revealed the dominant role of organo-mineral associations in the stabilization of nitrogen in the investigated soil.

1. Introduction

Litter decomposition and mineralization of soil organic matter are two of the main sources of bioavailable nitrogen (N) in forest soils and thus play a key role in the nutrition of forest trees. ^{15}N labeling techniques are frequently used to study N transformation and partitioning processes in forest soils. $^{15}NH_4^+$ or $^{15}NO_3^-$ have been applied in most experiments using isotopes for studying N dynamics in forest soils, because the use of inorganic N allows a rapid and localized investigation of the N partitioning between soil, plants, and microbial biomass (Preston and Mead, 1994; Perakis and Hedin, 2001; Compton and Boone, 2002). The application of ^{15}N-labeled litter material facilitates the identification of N release and redistribution of the original litter in the soil and thus provides insight into N cycling processes under less disturbed conditions (Högberg, 1997). Such an experimental setup with slow but long-term release of excess ^{15}N allows researchers to perform free-range experiments under natural conditions. Hence, this method is well suited to investigate long-term N processes and allocation. However, this technique is used only rarely, because both the production of the labeled residues and the long-term experiments are time and resource intensive.

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Most studies using labeled organic material investigated N processes in agricultural systems (Haynes, 1997; Kölbl et al., 2006; Wessels Perelo et al., 2006), whereas a few studies traced the fate of beech leaf litter N from decomposition in broadleaf forest ecosystems to its distribution in the plant–soil system (e.g., D'Annunzio et al., 2008). Zeller et al. (2000, 2001) were the first to investigate the decomposition of ^{15}N-labeled beech litter in beech forests with different soils and humus forms over three years. They showed that N released from the labeled litter was proportional to mass loss and that about 50% of the litter-derived N reached the topsoil (0–30 cm) after three years of litter decomposition. Furthermore, they observed a high variability for both N release and incorporation into the soil among forest sites, depending strongly on the soil type (Cambisol and Leptosol) and humus form (moder and mull-moder). Zeller and Dambrine (2011) compared the potential mineralization of the total soil organic N with that of recent litter-released N at the same sites after 4–5 years. They identified coarse particulate organic matter as the primary source of mineral N in topsoils of three beech forests. Aside from classic litterbag studies observing litter degradation (Olson, 1963), only Hatton et al. (2012) examined the transfer of forest leaf litter N in the mineral soil and its density fractions. They studied the incorporation of litter-derived N at 4, 8 and 12 years and showed that ~40% of the litter-derived N was present in soil organo-mineral associations after 4 years, suggesting a rapid incorporation at earlier stages of litter decomposition. Whereas C and N release and enrichment in soil organic matter and microbial biomass by litter decomposition have been already described (Bird and Torn, 2006; Fahey et al., 2011; Mambelli et al., 2011), the transfer of litter-derived N to soil organo-mineral associations remains unexplored for early stages of litter degradation.

To study the decomposition and distribution of ^{15}N-labeled mustard litter in cropland soils, Kölbl et al. (2006) applied a combined density and particle size fractionation procedure. This method allows the isolation of soil organic matter (SOM) fractions that are associated with particles of diverse size, structure, and functions defined by different specific stabilization mechanisms. These measurable SOM fractions represent functional SOM pools that have different roles in SOM turnover concerning rate and stability (von Lützow et al., 2007; Sollins et al., 2009). Physical fractionation is based on the concept that the organization of soil particles plays a key role in SOM dynamics, because bioavailability is a prerequisite for decomposition. Density fractionation is applied to separate the light fraction (LF), which is not firmly associated with soil minerals, from the heavy fraction. The free light fraction (fLF) is mostly related to primary litter input, whereas in soil aggregates occluded light fraction (oLF) is mainly affected by the aggregate turnover of the particular soils (Six et al., 1999). The heavy fraction contains organo-mineral associations in which SOM is more processed than in the LF, which contains less decomposed plant and animal residues (Christensen, 1992). The separation of the SOM into different functional fractions enables the study of major pathways of N stabilization processes, such as chemical recalcitrance, spatial inaccessibility, and the mineral association of SOM (von Lützow et al., 2006). Moreover, the combination of soil fractionation and labeling techniques in SOM studies (Mueller et al., 2009) enables a direct and rapid investigation of the decomposition and partitioning processes of SOM in the soil.

We used the application of ^{15}N-labeled beech litter in a submontane European beech forest (*Fagus sylvatica* L.) together with a combined particle size and density fractionation procedure using sodium polytungstate to investigate the incorporation and partitioning of litter-derived N in different functional SOM fractions in a clay-rich Rendzic Leptosol. This soil with mull humus form is characterized by high bioturbation through the activity of earthworms (Green et al., 1993; Zanella et al., 2011). Our aim was to identify the extent of N stabilization in such soils and their functional SOM fractions including fLF, organic matter occluded by aggregation, and organo-mineral associations. Transfer rates for the single functional pools were estimated using a complete dataset of three consecutive years. The experimental setup was designed to be adapted to the mull humus form and thus specifically allowed free access for macro fauna.

2. Materials and methods

2.1 Site characteristics

This study was conducted at the Tuttlingen Research Station (8°45′E, 47°59′N) in the Swabian Jura, a low mountain range in southwestern Germany. Dannenmann et al. (2008, 2009) previously described the study site which receives low atmospheric N deposition (<10 kg N ha^{-1} year^{-1}). The area's cool-moist climate is characterized by a mean annual air temperature of 6.5 °C and precipitation of 854 mm per year. The experimental plots of the present study were set up on an even steep slope (approx. 25° inclination) exposed to the northeast, at an elevation of approximately 800 m above sea level. European beech (*F. sylvatica*) is the main species, accounting for more than 90% of all the approximately 90-year-old trees (Dannenmann et al., 2009).

According to the International Union of Soil Sciences Working Group WRB (2007), the soil is classified as Rendzic Leptosol (Sceletic) developed on straight bedded Jurassic limestone and marls. Soil profiles are thin, developed on weathered parent rock enclosing approximately 45% gravel and stones. The Ah horizon at the study site is characterized by organic carbon (OC) contents of 64.1 ± 1.9 g kg^{-1}, N contents of 4.8 ± 0.1 g kg^{-1}, C/N ratios of 13.3 ± 0.2, and pH values of 5.7 ± 0.2. The clay content of the soil is high (70.1 ± 1.3%), whereas silt (26.8 ± 1.7%) and sand (3.2 ± 1.0%) contribute to soil texture to a minor degree. The mineralogy of the larger grain sizes is dominated by quartz; the clay fraction is relatively enriched in phyllosilicates, esp. irregular mixed-layer illite–smectite and kaolinite (Fig. 1). The bulk density of the Ah horizon is 0.8 g cm^{-3} resulting in an amount of 40 kg m^{-2} in 0–5 cm topsoil depth. The Ah horizon of the investigated Rendzic Leptosol located below the mull litter layer (FAO, 2006) is characterized by organo-mineral aggregates resulting from ingesting and mixing activities of earthworms (Ponge and Ferdy, 1997; Zanella et al., 2011).

2.2 Experimental design

In April 2008, three 2 m × 2 m plots were selected from a backslope with different relative altitudes with distances of at least 20 m. The litter layer was removed from each plot and replaced by an equivalent amount of ^{15}N-labeled beech litter (500 g litter m^{-2}). We thereby applied 237.48 mg ^{15}N to each plot. The labeled beech litter was evenly distributed on the plot surface and then fixed on top with a nylon net (1.5-cm mesh size) to avoid wind dispersal and slipping. The nylon net was fastened at the border with small wooden pales. In contrast to litterbag studies, this experimental design gives macro fauna access to the litter and allows for incorporation of the litter material into the mineral soil, characteristic for sites with mull humus form.

The ^{15}N-labeled beech litter was produced by spraying ^{15}N-labeled urea on the foliage of a 10-year-old beech stand in a natural regeneration area on calcareous soil (Calcisol) at Puvenelle near Pont-à-Mousson, France, in late summer of three subsequent years (Zeller et al., 1998). Senescent leaves were collected by hand from the labeled trees in November, dried at 30 °C, and stored until they were used as leaf litter in this experiment. This method achieved an

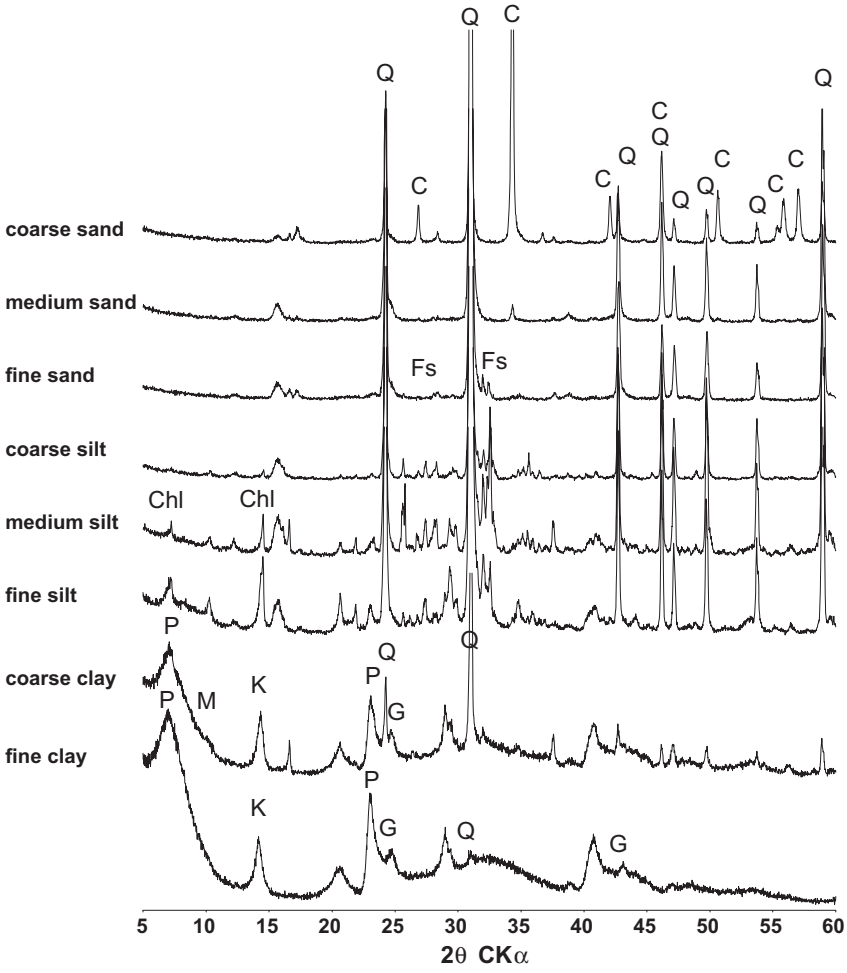

Fig. 1. XRD analysis of particle size classes. C = calcite, Chl = chlorite, Fs = feldspar, G = goethite, K = kaolinite, M = mica, P = other phyllosilicates, undifferentiated (esp. mixed-layer illite–smectite), Q = quartz.

^{15}N enrichment of 0.80520 ^{15}N atom% excess. The C/N ratio of the labeled litter (34.6 ± 0.4) was larger than that of the original beech litter removed from the plots (31.8 ± 1.3).

2.2.1 Litter sampling

Samples of ^{15}N-labeled beech litter were taken on the day of distribution (0 d) and again in May 2008 (21 d), June 2008 (70 d), September 2008 (140 d), June 2009 (428 d), September 2009 (507 d), and September 2010 (876 d). At each sampling, three litter samples were collected from an area of approximately 0.02 m^2 in each plot and the samples from each plot were mixed, resulting in three litter samples per sampling date. Collected litter samples were air-dried and homogenized using an ultra-centrifugation mill (ZM 100, Retsch, Haan, Germany) prior to chemical analysis.

2.2.2 Soil sampling

Before ^{15}N-labeled beech litter was distributed, reference samples were obtained by using a root auger (80-mm diameter) to collect three soil cores (0–10 cm) from spots with a distance of at least 1 m per experimental plot. Further soil samples were taken at days 21, 70, 140, 428, 507 and 876 of the experiment by using the same method, resulting in a total of nine soil samples per sampling date. Boreholes were refilled with quartz sand to minimize water disturbance. Each borehole was marked with sticks to prevent a second sampling from the same borehole. For analyses, the core was divided into two subsamples (0–5 cm and 5–10 cm). Each subsample was air dried and sieved to a particle size < 2 mm.

2.3 Physical fractionation design

To obtain data reflecting N stabilization in different SOM pools, we separated bulk soil samples into seven SOM soil fractions. Fractionation was performed on the samples harvested at the outset of the experiment and in autumn of three consecutive years, namely in September 2008, 2009, and 2010 (i.e., after 140, 507, and 876 d).

Bulk soil samples of 0–5 cm depth were subjected to a combined density and particle-size fractionation. Twenty grams of air-dried soil material (< 2 mm) were saturated with 200 ml of sodium polytungstate solution with a density of 1.7 g cm^{-3} and were allowed to settle overnight. Preliminary tests with this soil showed that this cut-off best separated plant residues from mineral soil. The floating fLF was extracted by sucking via a water jet pump. The fLF was washed several times with deionized water to remove the sodium polytungstate. To disrupt soil aggregates and liberate the occluded organic matter, the remaining residual was dispersed ultrasonically (Sonopuls HD 2200, Bandelin, Berlin, Germany; VS 70 T Sonotrode Ø 13 mm, at a power level of 70%) with a liquid coverage of 1.5 cm for 20 min, resulting in an energy input of 450 J ml^{-1}. The energy input was tested before to avoid disruption

of coarse LF and redistribution along with aggregate disruption. Subsequently, the samples were centrifuged (30 min at: 2.9×10^3 *g*) to separate the oLF from the denser organo-mineral pellet. The supernatant containing the oLF was passed through a sieve of 20-μm mesh size to obtain the oLF > 20 μm and oLF < 20 μm. The oLF < 20 μm was pressure filtrated, and both oLFs were washed several times with deionized water to eliminate excessive salt. To remove the salt from the remaining dense organo-mineral pellet, this fraction was centrifuged several times with deionized water until the fine clay fraction appeared in the supernatant. The dense organo-mineral pellet was then wet-sieved with a sieve of 20-μm mesh size to separate the sand and coarse silt fraction (> 20 μm). All material in the heavy fraction measuring > 20 μm was defined as the sand fraction. The organo-mineral soil fraction < 20 μm was separated into medium and fine silt (2–20 μm) and clay (< 2 μm) fractions by sedimentation. The size class 2–20 μm was referred to as the silt fraction. The fine clay (< 0.2 μm) fraction was obtained by centrifuging the clay fraction several times for 18 min at 4.0×10^3 *g* and collecting the supernatant until it stayed clear, followed by pressure-filtration of the collected supernatant. The residual sediments containing the coarse and medium clay (0.2–2 μm) were centrifuged with deionized water until the electrical conductivity of the supernatant dropped below 10 μS cm^{-1}. Each fraction was freeze-dried and weighed in order to determine the mass distribution based on the initial weight portion.

2.4 Chemical and physical analyses of bulk soil and soil fractions

For chemical analyses, bulk soil samples and soil fractions were homogenized using a vibrating ball mill with zircon-grinding tools (Pulverisette 23, Fritsch, Idar-Oberstein, Germany). For the subsoil samples (5–10 cm), the bulk soil was only analyzed for the last two samplings. The subsoil was not fractionated because results from previous studies indicate that only small amounts of ^{15}N reached that depth (Preston and Mead, 1995; Swanston and Myrold, 1997; Zeller et al., 2000; Zeller and Dambrine, 2011). Determination of total carbon (TC), N, and ^{15}N was performed in duplicate with an isotope ratio mass spectrometer (Delta V, Thermo Electron Corporation, Dreieich, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milan, Italy) at the Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Soil Ecology in Neuherberg. The ^{15}N-values were corrected at intervals using a lab standard (Acetanilid) that was part of every sequence. The standard was used in different weights to determine the isotope linearity of the system. The lab standard was calibrated against different international ^{15}N-standards from IAEA (e.g., IAEA N1, IAEA N2, USGS 40). The samples were free of carbonates so that the TC concentration equaled the OC concentration.

Analysis of bulk soil texture was conducted according to the method described by Mueller and Kögel-Knabner (2009). Organic matter was broken down using 30% H_2O_2 prior to texture analysis and X-ray diffraction (XRD). Clay mineralogy was assessed by XRD after Moore and Reynolds (1989). XRD was performed on random powder samples (Co-Kα; diffractometer PW1830, Philips, Alemlo, Netherlands) from all particle size fractions.

Soil pH was determined with a pH measuring instrument (WTW 197i, Weilheim, Germany) in 0.01 M $CaCl_2$ at a soil-to-solution ratio of 1:2.5 using a glass electrode (Hamilton, Höchst, Germany).

2.5 Data presentation and statistical analysis

^{15}N values are presented in ^{15}N atom%. Values of ^{15}N atom% excess were calculated by subtracting natural abundance ^{15}N atom% of the respective fractions of unlabeled soil (sampling after 0 d) from the enrichments obtained from the labeled fractions. To estimate the excess values in μg per kg soil, the mass distribution, N concentrations, and ^{15}N concentrations were multiplied for each fraction.

Mean values and standard errors were calculated using Microsoft Excel (ver. 14.0; Microsoft, Redmond, WA, USA). Further statistical analyses were carried out with SPSS (ver. 19.0; IBM, Ehningen, Germany). Figures were created either by Sigmaplot 11.0 (Systat Software GmbH, Erkrath, Germany) or Xact (ver. 8.05f, SciLab, Hamburg, Germany). Data were analyzed for homogeneity of variances by applying the Levene test, and the analysis of normality was performed using the Shapiro–Wilk test. Because a normal distribution was not guaranteed for the whole dataset, we tested for significant differences ($p < 0.05$) between the groups by applying the Kruskal–Wallis and Mann–Whitney tests. Because the Kruskal–Wallis tests showed no differences between the three plots within bulk soil and within soil fractions, we considered all nine samples as replicates at every occasion.

3. Results

3.1 Distribution of mass, OC, and N in soil fractions

3.1.1 Light fractions

Significant differences were found in mass concentrations, with the fLF varying between 11.3 and 59.0 mg (g soil)$^{-1}$ and the oLF < 20 μm ranging from 6.1 to 35.1 mg (g soil)$^{-1}$, respectively (Table 1). The oLF > 20 μm revealed significant differences in the OC concentrations, which ranged from 399.6 to 449.8 mg g^{-1}, and in the N concentrations, which varied between 13.9 and 15.7 mg g^{-1}. The contribution of the LFs to bulk soil OC exceeded 23%, whereas only 11–23% of total bulk soil N was stored within these three fractions. Except for the samples collected after 140 d, OC and N concentrations were clearly higher in the oLF > 20 μm than in any other fraction. With regard to the LFs, the results showed no obvious trends over the entire period investigated.

3.1.2 Organo-mineral fractions

The mass distribution of the organo-mineral fractions showed no variation over time. The clay-sized fractions accounted for nearly 50% of the bulk soil mass (Table 1), followed by the silt and sand fractions. The mass contribution of the sand fraction was larger in the fractionated soil (10%) compared to the measured soil texture (3%), where the clayey grain sizes accounted for 70%. In comparison to the samples from the fractionation method, the samples from the texture analysis were treated with H_2O_2 to destroy the organic matter. This altered the mass percentages and increased the amounts of grain sizes with comparatively little organic residues.

The lowest concentrations of N and OC were detected in the sand-sized fraction, whereas the coarse clay fraction showed the highest concentrations. The contribution of N and OC from the fractions to the bulk soil showed that the clay-sized fractions accounted for 64–75% of N and for more than 41% of OC, respectively (Table 1). We observed the highest N contents in each fraction at the outset of the experiment. A narrowing C/N ratio with decreasing particle size from the sand-sized fraction to the fine clay fraction was calculated (Table 1). We did not observe a trend in the C/N ratios of the individual fractions over the period of the experiment. This relationship suggests the stability of each fraction, especially the organo-mineral fractions.

Because the recovery rate of N summed over all seven fractions (Table 1) after the soil fractionation procedure amounted to 90% of the untreated bulk soil, we assume that a small amount of soluble N was extracted by the sodium polytungstate solution and subsequently discarded.

Table 1
Mass distribution, organic carbon (OC), and nitrogen (N) concentrations in soil organic matter (SOM) fractions per fraction and bulk soil and the contribution of N and OC of a specific fraction to the bulk soil depending on sampling date. Data are mean values with standard errors for nine replicates. Significant differences between different harvests of a single SOM fraction are indicated by lowercase letters ($p < 0.05$).

fraction	days	mass	N			OC			C:N
		mg [g bulk soil]$^{-1}$	mg [g fraction]$^{-1}$	mg [g soil]$^{-1}$	% of bulk soil	mg [g fraction]$^{-1}$	mg [g soil]$^{-1}$	% of bulk soil	
fLF	0	15.6 ± 3.3 bc	10.9 ± 0.5 b	0.2 c	3.4 c	352.2 ± 16.1 b	5.3 c	8.5 c	32.2 a
	140	59.0 ± 8.2 a	14.0 ± 0.6 a	0.9 a	16.9 a	346.1 ± 18.7 b	21.3 a	31.0 a	24.7 b
	507	25.6 ± 4.1 b	13.3 ± 0.7 a	0.3 b	7.8 b	372.8 ± 13.6 ab	9.6 b	17.1 b	28.1 a
	876	11.3 ± 1.4 c	13.3 ± 0.3 a	0.2 c	3.4 c	383.1 ± 3.4 a	4.3 c	8.5 c	28.8 a
oLF > 20	0	11.3 ± 2.3 ab	13.9 ± 0.4 b	0.2 ab	3.1 ab	449.8 ± 7.8 a	5.1 ab	8.1 b	32.4 a
	140	7.3 ± 1.3 b	14.4 ± 0.5 b	0.1 b	2.2 b	439.2 ± 10.3 a	3.3 b	4.9 b	30.6 a
	507	10.7 ± 1.0 a	14.9 ± 0.3 b	0.2 a	3.6 a	399.6 ± 2.8 c	4.3 a	7.8 a	26.8 b
	876	12.0 ± 1.4 a	15.7 ± 0.3 a	0.2 a	4.3 a	416.5 ± 3.7 b	5.0 a	9.9 a	26.5 b
oLF < 20	0	12.0 ± 2.1 bc	13.4 ± 0.6 ab	0.2 bc	3.3 b	271.0 ± 11.1 a	3.3 bc	5.8 b	20.2 ab
	140	6.1 ± 1.5 c	15.3 ± 0.8 a	0.1 c	2.4 b	278.4 ± 12.9 a	1.7 c	3.9 b	18.2 b
	507	35.1 ± 8.9 a	12.3 ± 0.4 b	0.4 ab	10.4 a	267.1 ± 12.2 a	9.2 ab	19.7 a	21.7 a
	876	21.7 ± 3.5 ab	13.1 ± 0.5 b	0.3 a	6.5 a	278.5 ± 7.7 a	5.9 a	12.8 a	21.2 a
sand	0	107.4 ± 0.0 b	0.5 ± 0.0 a	0.1 a	1.0 a	8.3 ± 0.0 a	0.9 a	1.5 a	17.5 b
	140	107.1 ± 5.5 ba	0.5 ± 0.1 ab	0.1 ab	1.3 ab	10.2 ± 1.9 a	1.1 ab	2.0 a	18.7 a
	507	108.7 ± 2.0 a	0.5 ± 0.1 ab	0.1 a	1.3 ab	8.6 ± 1.4 ab	0.9 ab	1.7 a	16.8 b
	876	106.3 ± 2.5 ab	0.4 ± 0.0 b	0.0 b	0.9 b	6.3 ± 0.5 b	0.7 b	1.3 a	16.8 b
silt	0	253.0 ± 0.3 a	3.9 ± 0.2 a	0.9 a	18.5 a	63.7 ± 1.4 a	15.6 a	25.9 a	16.5 ab
	140	260.0 ± 6.3 a	2.5 ± 0.2 b	0.7 b	13.6 b	43.9 ± 3.3 b	11.4 b	18.3 b	17.5 a
	507	250.1 ± 2.4 a	1.7 ± 0.1 c	0.4 c	9.9 c	26.9 ± 3.1 c	6.8 c	12.5 c	15.6 b
	876	256.0 ± 1.8 a	1.9 ± 0.1 c	0.5 c	10.6 bc	31.4 ± 3.2 c	8.0 c	15.7 bc	16.9 ab
coarse clay	0	225.0 ± 0.0 a	9.3 ± 0.2 a	2.1 a	42.3 a	91.9 ± 0.7 a	20.7 a	34.4 ab	9.9 a
	140	221.3 ± 5.5 a	8.3 ± 0.3 b	1.8 b	37.9 b	79.9 ± 5.4 b	17.6 b	28.0 c	9.7 a
	507	229.7 ± 6.4 a	7.5 ± 0.2 c	1.7 b	39.1 b	71.9 ± 2.9 b	16.5 b	30.4 bc	9.7 a
	876	223.9 ± 5.7 a	8.0 ± 0.2 bc	1.8 b	40.8 ab	77.6 ± 3.1 b	17.4 b	34.5 a	9.7 a
fine clay	0	242.7 ± 0.0 ab	5.8 ± 0.1 a	1.4 a	28.4 ab	40.2 ± 0.8 a	9.7 a	16.2 ab	6.9 a
	140	238.6 ± 6.1 bc	5.3 ± 0.1 b	1.3 bc	26.2 b	34.6 ± 1.4 c	8.3 b	13.3 c	6.5 b
	507	230.7 ± 9.2 c	5.4 ± 0.1 ab	1.2 b	28.7 b	33.1 ± 1.0 cb	7.6 b	14.2 bc	6.1 c
	876	258.8 ± 5.6 a	5.7 ± 0.1 ab	1.5 ac	33.7 a	35.4 ± 1.1 b	9.1 a	18.2 a	6.2 ac

fLF = free light fraction.
oLF > 20 = occluded light fraction > 20 μm.
oLF < 20 = occluded light fraction < 20 μm.

3.2 ^{15}N in litter, bulk soil, and soil fractions

The ^{15}N enrichment of the labeled beech litter declined quickly after litter application, especially during the first vegetation period, and was much slower thereafter (Fig. 2). After 876 d, ^{15}N in beech litter had only 16% of the initial ^{15}N atom% excess enrichment. This decrease indicated a release of the initial litter N besides diluting effects by natural, unlabeled litterfall.

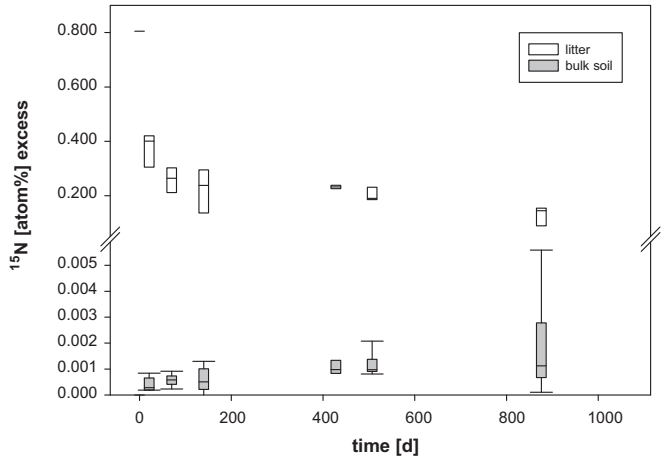

Fig. 2. ^{15}N excess of labeled beech litter and bulk soil. Box plots represent three replicates of each litter sample and nine replicates of bulk soil (0–5 cm depth) samples.

A significant increase in bulk soil ^{15}N atom% excess was detected 140 d after litter application. The ^{15}N natural abundance of the bulk soil (0–5 cm) was 0.36662 atom%. The ^{15}N atom% excess values increased consistently to 0.00184 atom% after 876 d, but the samples showed a large variability in ^{15}N atom% (Fig. 2). Bulk soil samples from a depth of 5–10 cm showed a slight enrichment of 0.00092 atom% excess after 507 d and 0.00068 atom% excess after 876 d (data not shown).

The fLF showed the highest ^{15}N enrichment of the soil fractions at each sampling date, except after 140 d (Fig. 3). From that day onward, it was significantly different from the natural abundance. Although the increase of the ^{15}N atom% excess of oLF > 20 μm was delayed, its ^{15}N signature was also significantly altered after 140 d. In comparison to the LFs, the development of the ^{15}N signature in atom% in the organo-mineral fractions showed a much smaller increase. All organo-mineral fractions had increased significantly after 140 d, but they changed little thereafter. Both clay fractions behaved similarly concerning the development in ^{15}N enrichment (Figs. 3–5); only the absolute values of total OC and N differed (Table 1).

9.4 μg ^{15}N (kg soil)$^{-1}$ were found in the fLF 140 d after litter application (Fig. 4). This value more than doubled by the following autumn (507 d) to 23.5 μg ^{15}N (kg soil)$^{-1}$ and reached 27.0 μg ^{15}N

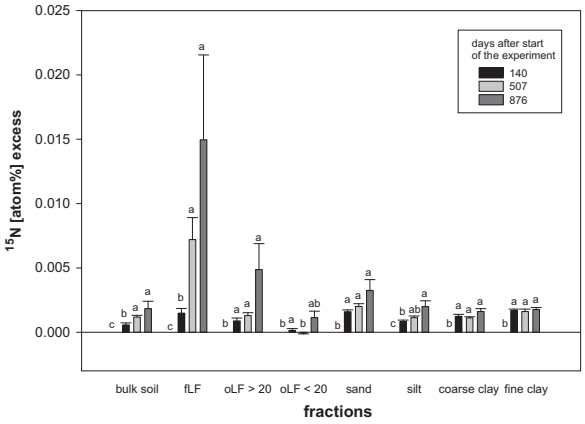

Fig. 3. ^{15}N excess in atom% in soil organic matter (SOM) fractions. Data are the mean of nine replicates, with error bars representing standard errors. Significant differences in ^{15}N excess between different harvests of a single SOM fraction are indicated by lowercase letters ($p < 0.05$). The first letter represents the first harvest at 0 d (i.e., natural abundance).

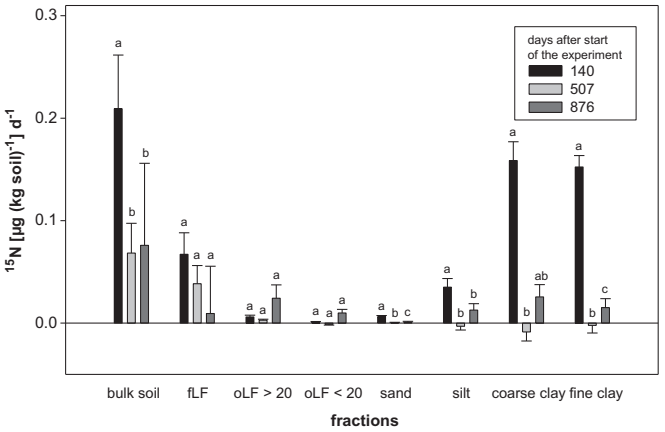

Fig. 5. Rates of net ^{15}N excess accumulation in µg (kg soil)$^{-1}$ d^{-1} in bulk soil and soil organic matter (SOM) fractions calculated as daily averages from intervals between the sampling dates ($n = 9$). Error bars indicate standard errors. Significant differences between different harvests of a single SOM fraction are indicated by lowercase letters ($p < 0.05$).

(kg soil)$^{-1}$ after 876 d. The ^{15}N excess in the oLF > 20 µm increased from 0.8 µg ^{15}N (kg soil)$^{-1}$ after 140 d to 10.8 µg ^{15}N (kg soil)$^{-1}$ after 876 d. The oLF < 20 µm fraction was at first ^{15}N-depleted, but it showed an excess of 3.4 µg ^{15}N (kg soil)$^{-1}$ after 876 d. Litter-derived ^{15}N increased significantly in all organo-mineral fractions within the first 140 d and did not follow any trend thereafter. The contribution of mineral-associated ^{15}N to the bulk soil was higher than that of the LFs due to the high N concentration and the mass of these fractions. Their amount after 140 d followed the same trend as their enrichments in atom%. After 876 d, 54.6 µg ^{15}N (kg soil)$^{-1}$ was found in the clay fractions (Table 2).

We calculated mean transfer rates from the litter to the mineral soil by dividing the excess in µg per kg soil for every fraction by the number of days between the sampling dates assuming constant rates. The mean rates of ^{15}N transfer from litter to the soil fractions ranged from losses of < 0.01 µg (kg soil)$^{-1}$ d^{-1} (coarse clay) to yields of 0.16 µg (kg soil)$^{-1}$ d^{-1} (coarse clay) over the course of the experiment (Fig. 5). The rates of ^{15}N input to each fraction showed a distinct variation over time: Whereas the organo-mineral fractions revealed a significant decline after the first 140 d, the oLF rates rose only in the period between 507 and 876 d.

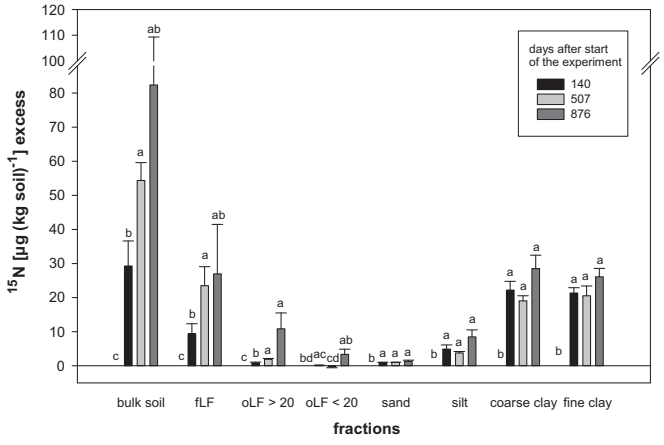

Fig. 4. ^{15}N excess in µg (kg soil)$^{-1}$ in bulk soil and soil organic matter (SOM) fractions ($n = 9$). Error bars indicate standard errors. Significant differences between different harvests of a single SOM fraction are indicated by lowercase letters ($p < 0.05$). The first letter represents the first harvest at 0 d.

We studied the concept of N dynamics by tracking our ^{15}N label as a specific cohort through seven SOM fractions. Herby, the ^{15}N tracer offered a snapshot of the status of a single cohort of litter-derived N. Thus, differences in ^{15}N tracer and total N distributions among the fractions should show where the litter-derived N cohort was favorably incorporated or retained over time. The distribution of the ^{15}N showed high values in the fLF compared to total N, which means that the litter-derived N was preferentially found there compared to all fractions (Fig. 6). We observed two further trends over the course of the experiment: (1) 140 days after litter application a higher percentage of ^{15}N compared to bulk soil N was found in the fine clay fraction. This high enrichment of ^{15}N in the fine clay fraction declined subsequently and (2) the recovery of ^{15}N in the oLFs was delayed.

By summing up the seven fractions in the upper 0–5 cm of the soil (Table 2), the recovery of litter-derived ^{15}N tracer revealed 4.0% after 140 d, nearly 4.7% after 507 d and 7.1% after 876 d. Regarding the absolute concentrations, more than 60% of the total ^{15}N label recovered was found in the organo-mineral fractions at every sampling date. In the bulk soil of the subsoil horizon (5–10 cm depth), we recovered another 2% of litter-derived ^{15}N tracer after 876 d.

4. Discussion

4.1 ^{15}N in bulk soil: recovery from litter application

The litter-derived N of our study accumulated mainly in the top mineral soil, progressively increasing the topsoil's ^{15}N signature (Fig. 2). Litter-derived N is principally found in the upper centimeters of the Ah horizon of different forest ecosystems over the first decade after litterfall (Zeller et al., 2000, 2001; Zeller and Dambrine, 2011; Hatton et al., 2012). After 876 d, we recovered 9% of the added label in the 0–10 cm soil horizon. Other free range studies showed similar results reporting the distribution of ^{15}N-labeled beech leaf litter within different compartments of the ecosystem over time (Hatton et al., 2012). Mueller et al. (2009) showed a decreasing ^{15}N enrichment trend with increasing soil depth due to less fresh SOM input in deeper layers.

The comparably slight ^{15}N enrichment of the mineral soil in our experiment can be ascribed to the small enrichment of the labeled litter. The incorporation of residues into the soil via bioturbation and without any artificial mixing showed a high degree of variability (Fig. 2). The increase in the standard errors with time (Figs. 3

Table 2
Recovery of ^{15}N excess in labeled litter in soil organic matter fractions of the 0–5 cm soil layer expressed in μg (kg soil)$^{-1}$ and as a percentage. Significant differences between different harvests of a single soil organic matter fraction are indicated by lowercase letters ($p < 0.05$).

fraction	μg ^{15}N (kg soil)$^{-1}$ excess			% of added ^{15}N excess in labeled litter		
	140 d	507 d	876 d	140 d	507 d	876 d
fLF	9.40 ± 2.94 b	23.50 ± 5.56 a	26.98 ± 14.51 ab	0.63	1.58	1.82
oLF > 20	0.85 ± 0.24 b	1.90 ± 0.26 a	10.85 ± 4.65 a	0.06	0.13	0.73
oLF < 20	0.16 ± 0.06 ab	−0.27 ± 0.32 b	3.37 ± 1.49 a	0.01	−0.02	0.23
sand	0.88 ± 0.16 a	1.01 ± 0.07 a	1.29 ± 0.35 a	0.06	0.07	0.09
silt	4.91 ± 1.18 a	3.77 ± 0.40 a	8.47 ± 2.05 a	0.33	0.25	0.57
coarse clay	22.21 ± 2.56 a	19.06 ± 1.49 a	28.49 ± 3.95 a	1.50	1.28	1.92
fine clay	21.33 ± 1.56 a	20.54 ± 2.86 a	26.11 ± 2.46 a	1.44	1.38	1.76
sum	**59.74 ± 6.18 a**	**69.51 ± 4.79 a**	**105.57 ± 27.40 a**	**4.02**	**4.68**	**7.11**

fLF = free light fraction.
oLF > 20 = occluded light fraction > 20 μm.
oLF < 20 = occluded light fraction < 20 μm.

and 4) reflected the heterogeneity of ^{15}N levels in the nine parallel samples that rose over time due to the dilution effects by natural unlabeled litter and the environmental conditions of the experiment. Haynes (1997) suggested that the low recovery of N respective to ^{15}N in studies of decomposition of residues was due to the dependence on environmental conditions among other factors. Gaseous and fluid losses of nitrate and dissolved organic nitrogen as well as subsamples harvested for analysis must be taken into consideration. Additionally, losses of litter material may occur due to edge effects of the slope, as a result of colluvial and eluvial losses. Finally, the plots were not litter-free by the end of the experiment, and some labeled material remained on the forest floor. However, the transfer rates to the bulk soil decreased with time (Fig. 5) because less labeled litter material was available. In total, the soil partitioning process demonstrated a dynamic consisting of (1) slow total litter decomposition and (2) incorporation by soil fauna.

4.2 Transition of ^{15}N from litter to organo-mineral associations via the light fractions

The ^{15}N enrichment in atom% in the fLF by 140 d after litter application indicated a rapid transfer of the applied fresh litter from the soil surface into the upper mineral soil horizon (Figs. 2 and 3). These mechanisms of translocation may be traced back to leaching effects (Lajtha et al., 2005; Fahey et al., 2011) and the high bioturbation by earthworms and probably also voles at the study site, as reflected by the mull humus form. The organic matter in the fLF was composed of particulate, partly decomposed plants, and to minor extent animal residues. The LFs functioned as a transitory pool between litter and mineral-associated organic matter, with turnover times between more rapidly decaying litter and more slowly decaying organic matter associated with organo-mineral fractions (Christensen, 2001). On every sampling day after the start of the experiment, the fLF showed higher ^{15}N enrichments than the oLFs, probably because the oLFs were physically protected from microbial decay due to spatial inaccessibility of the aggregates. Therefore, the input to this fraction was decelerated (Figs. 3–5). On the one hand, the litter-derived material degraded rapidly, but on the other hand the low ^{15}N enrichments in the oLFs displayed a slow aggregate turnover. This implies a high stability of aggregates in the studied soil and a rate for a reformation of aggregates of more than 1 year, because we did not observe any significant differences in the oLF < 20 μm during the 876-d experimental period. According to Christensen (2001) soil microaggregates, especially those < 20 μm, are rather stable over long periods, which may explain the steady state of ^{15}N enrichment in the oLF < 20 μm during the first harvests and the delayed increase of this fraction at 876 d (Fig. 3). This may be attributed to a long process of strong comminution and subsequent integration into the aggregate. Rasmussen et al. (2005) and McFarlane et al. (2012) moreover showed that oLF might be very old, and even older than the mineral-attached OC found in dense organo-mineral fractions by providing ^{14}C turnover times for oLF isolated from temperate (American) forests. The high values of ^{15}N excess in the fLF (Figs. 3 and 4) indicated no depletion of the easily decomposable litter source. From Figs. 3 and 4 we may assume that bioturbation kept delivering organic matter, but net ^{15}N excess accumulation rates in the fLF decreased (Fig. 5).

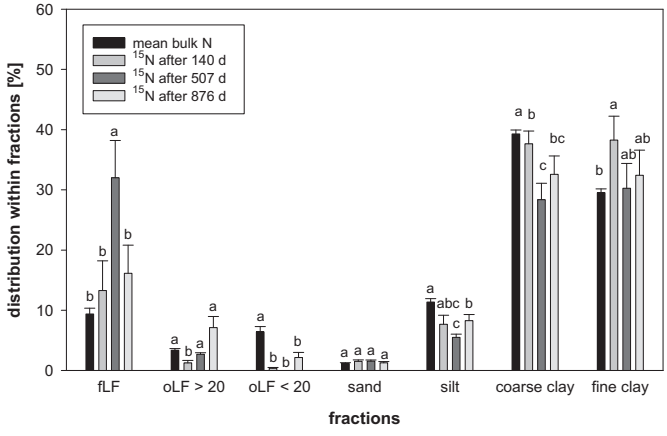

Fig. 6. N and ^{15}N distribution in soil organic matter fractions. Black bars represent the mean bulk N and gray bars the ^{15}N tracer ($n = 9$). Error bars indicate standard errors. Bars marked with different lowercase letters have significantly different distributions ($p < 0.05$).

4.3 Rapid recovery of ^{15}N in organo-mineral fractions

During the first vegetation period, the ^{15}N was rapidly transferred to the organo-mineral fractions (Fig. 5), especially the clay fractions. The following years brought less input, even losses in the second year. Based on earlier studies (Guggenberger and Kaiser, 2003; Lajtha et al., 2005), the assumption has been made, that two processes can be responsible for the fast increase in the ^{15}N enrichment of the organo-mineral fractions during the initial phase of decomposition, i.e. (1) leaching of ^{15}N and sorption to the mineral phase (Aita et al., 1997; Haynes, 1997; Hatton et al., 2012) and (2) incorporation by bioturbation (Aita et al., 1997; Hatton et al., 2012). As in previous investigations (e.g. Holub and Lajtha, 2004), it was shown that ^{15}N stored in the microbial biomass was the dominant pool of the ^{15}N recovery in the extractable soil pools, with a constant value of approximately 0.3% recovery over the experimental period (M. Dannenmann, personal communication). Continuous recycling, mediated by the microbial biomass, may

explain the steady ^{15}N recovery in the extractable pools (Holub and Lajtha, 2004), because no decrease with time due to unlabeled sources by dilution was detected. Several studies support the assumption that soil microbial biomass and its residues are mainly associated with the clay fractions (Jocteur Monrozier et al., 1991; Kandeler et al., 2000). In addition, the LFs increasing in ^{15}N excess enrichment throughout the whole period were able to provide ^{15}N in the form of plant debris, which was further transferred from plant fragments to organo-mineral associations with some time lag. These more decomposed products may contribute to the ^{15}N enrichment of the clay fractions during a second stage, and are therefore more important in the long-term view (Christensen, 1992). At 876 d after the start of the experiment, we still observed an incorporation of labeled material. The fine clay of our study was not saturated with N, as this fraction could retain proportionally even more of the ^{15}N tracer than total N (Fig. 6).

In similar leaf litter experiments, Hatton et al. (2012) showed that the formation of organo-mineral associations is a central mechanism for decadal- to longer-term preservation of litter-derived N in forest soils. They observed increases in the litter-derived N of heavy fractions at 4 years and even at 12 years after leaf litter application. Thus, we assume that the organo-mineral fractions were also stable in our experiment over three years and that their enrichment may further rise with time. Their sorption character and the inaccessibility may protect the litter-derived N from microbial activity, which consequently leads to a stabilization process (Kögel-Knabner et al., 2008; Hatton et al., 2012). Our detailed particle size fractionation allowed us to observe the unique importance of the clay fractions in terms of N partitioning, because their ^{15}N enrichment hardly changed after the first rapid pulse (Figs. 3–5). With regard to the organo-mineral fractions, this rapid flux may be generally important for the attachment of ^{15}N to organo-mineral associations over a medium time scale, because ^{15}N enrichment concentrations in atom% excess and μg per kg soil stayed constant and rates slowed after that first pulse (Figs. 3–5). Therefore, the clay fractions may act as a sink, with ^{15}N remaining in the system, because their pool sizes did not change significantly after 140 d. This conclusion was only valid for the recovered ^{15}N, while the fate for the remaining 90% stayed uncertain.

Although the ^{15}N atom% excess of sand and silt increased significantly after 140 d, these fractions played quantitatively minor roles in the turnover of the labeled litter material (Fig. 4) due to very low mass and N concentrations (Table 1). The rise in the ^{15}N atom% enrichment in the sand fraction can be ascribed to particles of particulate organic matter. Considering this, the fractionation method reached its limit in separating light material from the heavy fractions.

5. Conclusions

Our results revealed a specific response of the different SOM fractions in a highly aggregated clay-rich Rendzic Leptosol with a mull humus form.

During the experiment, ^{15}N transfer rates from litter slowed down in the fLF but even after three years one fourth of the recovered ^{15}N in the mineral soil was still present in the form of free plant debris incorporated by bioturbation. Plant debris occluded in aggregates occurred in substantial amounts at the final stage of the experiment only, demonstrating a delayed relocation of the ^{15}N tracer in oLFs due to a slow aggregate turnover. It may therefore act as a temporary storage of ^{15}N.

Incorporation of litter-derived ^{15}N in the mineral soil occurred mainly in the coarse and fine clay fractions, with an unexpectedly rapid transfer of ^{15}N completed within 140 d. ^{15}N in organo-mineral associations accounted for more than 60% of the total ^{15}N recovered, presenting a rapid stabilization of the recovered ^{15}N derived from leaf litter in the long-term pool. This result underlines the dominant role of organo-mineral associations for the stabilization of nitrogen in the investigated soil.

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Study II

Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions

by

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Prolonged summer droughts retard soil N processing and stabilization in organo-mineral fractions

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ABSTRACT

Prolonged summer droughts are projected to occur as a consequence of climate change in Central Europe. The resulting reduced soil water availability may lead to alterations in rates of soil processes such as nitrogen partitioning among soil organic matter fractions and stabilization within soil. To study the effect of climate change-induced drought on (1) the distribution of nitrogen among soil organic matter fractions and (2) nitrogen stabilization, we performed a space-for-time climate change experiment. We transferred intact plant–soil–microbe mesocosms of a Rendzic Leptosol with a young beech tree from a slope with northwestern exposure in southern Germany characterized by a cool-moist microclimate across a narrow valley to a slope with southwestern exposure with a warm-dry microclimate, which reflects projected future climatic conditions. A control transfer was also done on the northwest-facing slope within the same area of origin. We combined a homogenous ^{15}N labeling approach using ammonium nitrate with a physical fractionation procedure and chemical soil extraction protocols. Our aim was to follow the partitioning of ^{15}N in different soil organic matter fractions, i.e. light fractions, organo-mineral fractions, and extractable soil fractions including microbial biomass, ammonium, nitrate, and dissolved organic nitrogen. Within less than one growing season, we observed a modified partitioning of recently applied inorganic ^{15}N between different soil fractions in relation to drier summer conditions, with attenuated nitrogen turnover under drought and consequently significantly higher ^{15}N concentrations in the relatively labile light fractions. We ascribed this effect to a decelerated mineralization immobilization turnover. We conclude that prolonged summer droughts may alter the stabilization dynamics because the induced inactivity of microorganisms may reduce the transfer of nitrogen to stabilization pathways. A retarded stabilization in organo-mineral associations enhances the risk of nitrogen losses during extreme rainfall events, which are projected to increase in the 21st century predicted by future climate change scenarios for Central Europe
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1. Introduction

Climate change is expected to have a substantial impact on soil moisture and temperature conditions (Intergovernmental Panel on Climate Change IPCC, 2007), with enhanced frequency and duration of summer droughts (Kunstmann et al., 2004) in Central Europe due to increased evapotranspiration or shifts in precipitation patterns (Borken and Matzner, 2009). This will decrease soil water availability and may thus negatively affect growth, development, and competitiveness of beech forests in Central Europe, particularly on soils with low water retention capacity (Geßler et al., 2007). Furthermore, reduced soil moisture will decrease the uptake kinetics of mineral nutrients by beech trees and thus indirectly limit their growth (Geßler et al., 2004). This vulnerability to the

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increasing incidence of dry summers may hence have an impact on nitrogen (N) fluxes in forest soils. This research topic is important, because European beech forest ecosystems provide important economic and ecological services and dominate the potential natural forest vegetation in Central Europe.

Beier et al. (2008) and Emmett et al. (2004) investigated the response of the N cycle to global warming in European scrubland ecosystems. They found that net N mineralization was sensitive to soil moisture. The effects of drought on the immobilization of inorganic N were described by Compton and Boone (2002) in a ^{15}N labeling study that suggested that low soil moisture inhibited microbial uptake of inorganic N. Yet, few studies have examined how the altered conditions due to climate change will affect N dynamics. This might be due to the complexity of the N cycle arising from a multiplicity of N transformation processes and fluxes involving both organic and inorganic N compounds. Therefore, research on N cycling processes requires a general analysis of different organic and inorganic soil N fractions, including soil microbial biomass (MB-N), dissolved organic nitrogen (DON), ammonium (NH_4^+), and nitrate (NO_3^-) as well as total soil N present in organic and organo-mineral soil organic matter (SOM) fractions. Understanding these processes and their quantitative significance for N fluxes in forest ecosystems is an essential prerequisite for the identification and characterization of competitive mechanisms that determine N partitioning between organisms and soil fractions in a changing environment.

To investigate responses to climate change, Rennenberg et al. (2009) recommended studies using intact plant–soil systems that focus on simultaneous measurements of all major N fluxes including plant uptake and release of organic and inorganic N compounds. Borken and Matzner (2009) also called for further research on the sensitivity of microorganisms involved in the N cycle to drought stress. Most of the studies on microbial N turnover or plant N uptake in forest ecosystems determined N fluxes only after the plant–soil system was totally altered. Only a few studies (Ineson et al., 1998; Hart and Perry, 1999; Hart, 2006) have used soil or soil–plant transplants across climatic gradients, following the space-for-time approach to simulate climate change. This methodology has benefits over controlled laboratory or greenhouse studies in that ecosystem mechanisms are exposed to micro-environmental dynamics that are difficult to simulate in the laboratory (Shaver et al., 2000).

Therefore, we conducted an in situ ^{15}N tracing experiment with intact beech understory–soil systems in a typical European beech (*Fagus sylvatica* L.) forest in southern Germany growing on Rendzic Leptosol. We transferred intact plant–soil–microbe mesocosms each containing a naturally established young beech sapling from a northwest-facing (NW) slope (control) to a southwest-facing (SW) slope (drought) to assess the potential impacts of simulated climate change on soil N. Controls were transferred to the same altitude and elevation on the NW slope, which served as a model for current conditions with a cool-moist microclimate, whereas the warm-dry SW slope represented the climate change conditions in Central Europe (Geßler et al., 2004) as projected by Intergovernmental Panel on Climate Change IPCC (2007). Because the properties of the soil mesocosms and initial size of the beech saplings were similar, the results of this study can be directly related to the differing climatic factors. In order to investigate drought effects on N partitioning to soil and plant fractions of different stability and turnover times, we homogenously applied labeled $^{15}NH_4^{15}NO_3$ to the intact plant–soil–microbe mesocosms. We monitored N in different SOM fractions as well as MB-N, inorganic N, and DON over one growing season. Our main objectives were: (1) to quantify the distribution of the applied ^{15}N between different soil and plant N fractions under ambient control and simulated drought conditions, (2) to determine the mechanisms responsible for N stabilization within SOM fractions, and (3) to evaluate the effects of drought on N stabilization in soil.

We combined a labeling approach with a soil fractionation procedure, allowing for rapid investigation of SOM partitioning in functionally different SOM fractions (Kölbl et al., 2006; Mueller et al., 2009). This method enabled us to assess the stabilization chain including active/labile, intermediate, and long-term stabilized fractions (Bimüller et al., 2013) and the associated mechanisms of selective preservation due to recalcitrance, spatial inaccessibility, and interaction with mineral surfaces and metal ions (von Lützow et al., 2006).

Our approach was based on two hypotheses. First, decomposition is associated with high mineralization immobilization turnover (MIT), which in turn favors the stabilization of organic N in the form of microbial residues in organo-mineral associations. The MIT will be influenced by drought conditions. Second, the N in organo-mineral associations will be less stabilized under drought conditions, due to a lower microbial biomass level.

2. Materials and methods

2.1 Study site description

The experiment was conducted in a long-term ecological beech research forest near Tuttlingen on the Swabian Jura (Baden-Württemberg, Germany) at an elevation of 780 m above sea level (Bimüller et al., 2013). The clay-rich soil is classified as Rendzic Leptosol (Skeletic) according to the International Union of Soil Sciences Working Group WRB (2007). Bulk mineralogy by X-ray diffraction shows a relative enrichment of the clay fractions in phyllosilicates, especially irregular mixed-layer illite-smectite and kaolinite. Quartz dominates in the larger grain sizes (Bimüller et al., 2013). Soil profiles under the mull humus are shallow, underlain by gravel-rich layers of weathered bedrock or periglacial layers derived from limestone. At the field site near Tuttlingen, we took advantage of exposure-induced model ecosystems located on different slopes of a narrow valley, showing a cool-moist (NW aspect) and a warm-dry local climate (SW aspect) within a distance of < 1 km of each other. At both sites, beech is the dominant species, contributing $>$ 90% of the total basal area of all trees (Geßler et al., 2001). The cool-moist NW slope represented the present mesoclimatic circumstances typical of a beech stand in Central Europe (Geßler et al., 2004). The situation on the SW aspect was assumed to be representative of future climatic conditions with higher soil temperatures (1 °C) and summer droughts, as predicted by climate projections for Central Europe (Special Report on Emission Scenarios B1/A1B/B2 scenario range) for the 21st century (Geßler et al., 2004, 2007; Intergovernmental Panel on Climate Change IPCC, 2007). Additionally, the frequency of extreme weather events like heavy rainfall is projected to increase in the 21st century. Continuous measurements of meteorological and edaphic parameters have been carried out at both sites since 1999, showing an advanced soil development with higher fine earth content and less gravel or coarse fragments on the wetter NW slope. Rainfall does not vary significantly between the two sites across the valley (Geßler et al., 2001). Greater radiation on the SW slope, however, results in higher soil temperature and thus less water availability due to greater evapotranspiration (Geßler et al., 2004). A comprehensive dataset on soil microbial, tree physiological, and tree growth parameters has been collected (Geßler et al., 2004).

2.2 Experimental design

The experimental design of this field study consisted of an intact plant–soil–microbe mesocosm transfer simulating climate change conditions coupled with a ^{15}N labeling experiment. A summary of the soil management during the experimental period is listed in Table 1.

Table 1
Experimental management in the investigated period.

Date	Days after start of the experiment	Activity/soil management
July 2010		Transfer of intact plant–soil–microbe mesocosms from NW → NW and NW → SW
21 June 2011	0	Application of $^{15}NH_4^{15}NO_3$ label Sampling of unlabeled plant–soil–microbe mesocosms Soil sampling of labeled plant–soil–microbe mesocosms t1
28 June 2011	7	Setup of climate change treatment roof on SW slope Sampling of labeled plant–soil–microbe mesocosms t2
23 July 2011	32	Sampling of labeled plant–soil–microbe mesocosms t3
9 August 2011	49	Destruction of climate change treatment roof on SW slope
26 September 2011	97	Sampling of labeled plant–soil–microbe mesocosms t4

2.2.1 Transfer of intact plant–soil–microbe mesocosms

Thirty intact plant–soil–microbe mesocosms were re-sited to simulate climate change conditions. We used the cool-moist microclimate from the NW slope to model the climate of present-day conditions and the warm-dry microclimate from a nearby SW slope to simulate future climate change conditions. The latter simulated climate change conditions were intensified through the setup of a temporary translucent rain sheltering roof (27 June 2011 to 9 August 2011), which guaranteed a summer drought over six weeks. Trenches around the 1 m tall rain shelter avoided slope water. This approach enhanced the pronounced differences in soil temperature and soil moisture (Fig. 1) and allowed a free air flow and active radiation.

In summer 2010, stainless steel cylinders were manually driven into the soil of a preselected sampling area on the NW slope of the beech forest. The sampling area (10 m × 10 m) was in the middle of the homogenous slope (23°) and was selected after intensive drilling had revealed similar soil profiles in this area, with larger stones, periglacial layers, or weathered bedrock occurring at depths greater than 15–20 cm. The inner diameter of the soil cores was 162 mm and the length was 150 mm (2 mm wall thickness of cylinders). Each intact soil core was centered around a beech sapling of natural beech rejuvenation (approximately 2–3 years old) with a stem diameter of 2 mm and a height of 30 cm. This size of beech saplings had a root architecture that fit well within the volume of the soil stainless steel cylinders. Fifteen intact plant–soil–microbe mesocosms were transferred from the NW to the SW slope randomly from all universe mesocosms, which resulted in their experiencing an approximately 1 °C increase in mean annual soil temperature (5 cm depth) as well as significantly reduced soil moisture. Another 15 randomly chosen intact plant–soil–microbe mesocosms were transferred in situ at the same altitude and elevation on the NW slope as controls within the original preparation site. Three of the fifteen mesocosms were left unlabeled on each slope for measurement of natural $\delta^{15}N$ abundance. We transferred 10 additional mesocosms for monitoring soil temperature and soil moisture at 5 cm depth ($n = 5$ replicates each for control and drought transfers) using horizontally installed (through holes in the stainless steel cylinder walls) combined soil moisture/temperature probes (DECAGON EC-5 Decagon Devices, Inc., Pullman, Washington USA) that gathered data hourly. All mesocosms were destructively sampled in triplicates for each sampling date within the experimental unit of 2 m × 4 m on each site.

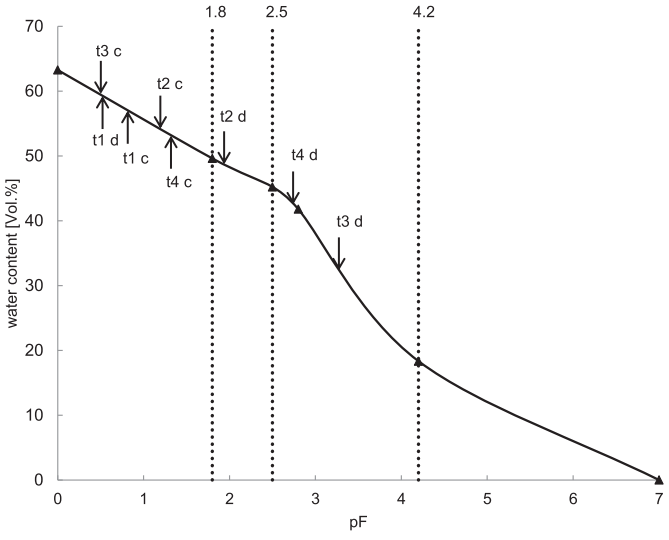

Fig. 1. Soil water potential of the Rendzic Leptosol used for mesocosms. Arrows indicate soil water potential at the sampling dates of control (c) and drought (d) treated soil mesocosms at the four time steps (t1–t4) of harvest.

After transfer, all intact plant–soil–microbe mesocosms on both slopes were irrigated with 500 ml (corresponding 23.7 l m^{-2}) of water to prevent drying or death of enclosed beech trees due to inevitable cutting of the roots. Subsequently, the mesocosms were left undisturbed in situ for almost an entire year to facilitate regeneration of the enclosed plant–soil–microbe system from the impacts of coring and trenching, and to allow acclimation to the new environmental conditions.

2.2.2 ^{15}N label application

After a pre-incubation period of 11 months, intact plant–soil–microbe mesocosms were labeled on 21 June 2011 with 130 ml of a label solution containing $^{15}NH_4^{15}NO_3$ at 99 atom% ^{15}N enrichment corresponding to an excess amount of 15.66 mg ^{15}N per soil mesocosm. The concentration of added N was 18.81 mg NH_4^+–N kg^{-1} soil dry weight (sdw) and 18.81 mg NO_3–N kg^{-1} sdw, corresponding to 450% and 250% of the natural background concentrations measured at the same time in unlabeled mesocosms, respectively. This labeling treatment increased the water content of the soil by approximately 5% of sdw of unlabeled mesocosms.

Before labeling, the mull litter layer was removed from the top of the soil cores. We developed a labeling pattern with 16 injections and three depths of 1, 3, and 6 cm following the intact plant–soil system isotope labeling approach of Wu et al. (2011). Additionally, 10 ml of label solution were homogenously spread on top of the soil surface. This labeling pattern was optimized for the soil of the experimental site in a pilot study that tested numerous injection patterns and amounts of injected label using Brilliant Blue FCF color dye (Sigma Aldrich, Taufkirchen, Germany). The pattern used was the best compromise between the conflicting requirements of homogenous three-dimensional labeling of the entire soil core and a minimal amount of water addition and leakage at the bottom of the cores. For the injections, we used commercial stainless steel side port cannulas that were adapted to our requirements. Paper calibers indicating injection patterns and depths were constructed to fit onto the cylinders to ensure reproducible labeling.

2.3 Sampling and sample preparation

Three of the 15 intact plant–soil–microbe mesocosms on each slope were harvested on each of the following dates: 21 June 2011

(t1: 0 days), 28 June 2011 (t2: 7 days), 23 July 2011 (t3: 32 days), and 26 September 2011 (t4: 97 days). Soil mesocosms were carefully dug out by hand and immediately processed. Soil was completely removed from the stainless steel cylinders and separated from the belowground biomass, and roots and gravel were removed by hand. Finally, the soil of each mesocosm was homogenized and mixed by hand in a field-moist state to ensure full mixing for a homogenous sample.

Fresh weight of the whole soil contained in the soil mesocosm was determined. A fresh soil subsample was extracted 1:1.3 (soil:solution) with 0.5 M K_2SO_4 immediately after sampling in the field laboratory and subsequently frozen for later analysis of extractable N and ^{15}N (MB-N, DON, NH_4^+, NO_3^-). A second subsample was fumigated with chloroform vapor for 24 h and then extracted with 0.5 M K_2SO_4 (Dannenmann et al., 2006). A third subsample was taken and immediately frozen to stop all processes. This frozen soil sample was used for further partitioning into respective SOM fractions and subsequent analyses of their total N concentration and ^{15}N enrichment.

During each sampling session, beech seedlings were carefully removed from soil mesocosm by hand, followed by separation of the plants into leaf, wood, and root material. Roots were separated by hand to obtain the living part, which was then washed with tap water and weighed. The fresh weight of each tissue was determined, and then samples were freeze-dried, ground manually in liquid nitrogen, and further milled with a vibrating ball mill with zircon-grinding tools (Pulverisette 23, Fritsch, Idar-Oberstein, Germany) to obtain a homogenous powder.

2.4 Physical fractionation design

In order to obtain the different organic and organo-mineral SOM fractions, freeze-dried bulk soil samples were sieved (< 2 mm) and subjected to combined density and particle-size fractionation according to Bimüller et al. (2013), Fig. 2. The fractionation procedure was specifically tested and adapted to this soil type. We obtained the following fractions: free light fraction (fLF), occluded light fraction > 20 μm (oLF > 20 μm), occluded light fraction < 20 μm (oLF < 20 μm), sand (2000–20 μm), silt (20–2 μm), and clay (< 2 μm).

2.5 Chemical and physical soil analyses

For chemical analyses, bulk soil samples and SOM fractions were finely ground using a vibrating ball mill with zircon-grinding tools (Pulverisette 23, Fritsch, Idar-Oberstein, Germany). Determination of total soil carbon (TC), N, and ^{15}N was subsequently performed in duplicate with an isotope ratio mass spectrometer (Delta V, Thermo Fisher Scientific, Bremen, Germany) coupled to an elemental analyzer (Euro EA, Eurovector, Milan, Italy). To examine the exchangeable bound ammonium in the clay fraction, we extracted a subsample of 200 mg of this fraction three times with 20 ml of a 2 M KCl solution each time. The extracts were measured photometrically at 655 nm (Spectronic 601, S/N 3612117004, Milton Roy, Ivyland, Pennsylvania, USA). We assumed that the water-extractable NH_4^+ was already leached by water from clay during the fractionation procedure. Inorganic carbon (IC) concentrations of bulk soil samples were determined using 7 ml of 4 M HCl and 2 ml deionized H_2O (08.53 Calcimeter, Eijkelkamp, Giesbeek, the Netherlands). The organic carbon (OC) concentrations of the bulk soil samples were calculated as the difference between TC and IC. The ^{15}N atom% excess was calculated by subtracting natural abundance values of unlabeled soil mesocosms from the values of the labeled fractions at each sampling date.

Gravimetric soil water content was determined using at least 100 g of fresh soil after drying at 105 °C until reaching a constant weight. Soil water tension of six undisturbed subsamples from the soil used for the mesocosms was determined using suction plates (pF-Laborstation 4251, ecoTech GmbH, Bonn, Germany) and a 15 bar ceramic plate (extractor no. #1500, Soil Moisture Equipment

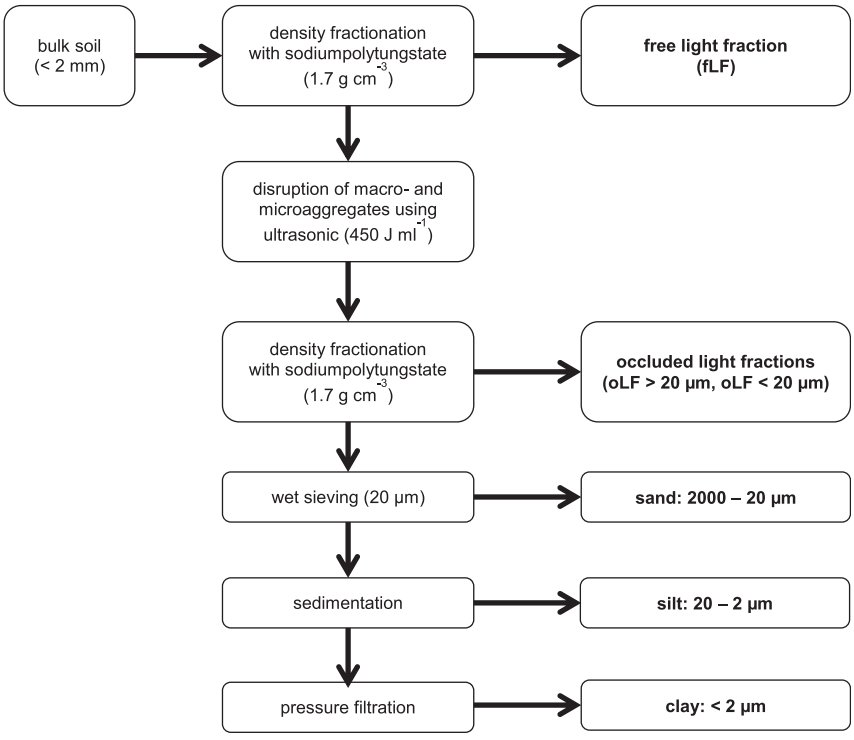

Fig. 2. Fractionation scheme.

Co., Santa Barbara, California, USA) coupled to a vacuum control system. Gravimetric soil water contents of the experiment were converted to volumetric soil water contents by multiplying by the bulk density. Prior to the analysis of the bulk soil texture of the soil used for the mesocosms, we removed carbonates with 1 M HCl, oxidized the organic matter using H_2O_2 (30%), and used a 0.025 M $Na_4P_2O_7 \cdot 10H_2O$ solution for particle dispersion. The fractions > 63 μm were separated via wet sieving, whereas those < 63 μm were determined using the X-ray attenuation method (Sedigraph 5100, Micromeritics Instrument Corporation, Norcross, Georgia, USA). We determined soil pH with a pH meter (WTW 197i, Weilheim, Germany) in 0.01 M $CaCl_2$ at a ratio of 1:2.5 (soil:solution) using a glass electrode (Hamilton, Höchst, Germany).

2.6 Compound-specific and ^{15}N isotope analysis of soil extracts

All extracts conducted in the field were frozen until further processing in the laboratory. Soil extracts were used for determination of NH_4^+ and NO_3^- concentrations in a commercial laboratory (Dr. Janssen, Gillersheim, Germany) as well as for determination of total chemically bound N and total organic C by a chemoluminescence detector (CLD-TOC) coupled to a C and N analyzer (multi C/N 3100, Jena Analytics, Jena, Germany). Dissolved organic nitrogen was calculated by subtracting inorganic N in soil extracts from total dissolved N. Microbial biomass C and N were determined with the chloroform-fumigation extraction method described by Dannenmann et al. (2009) and were calculated from the OC and total N release from fumigated soil compared to unfumigated control soil; no correction factors were applied for incomplete recovery of microbial C and N to gain conservative estimates of the active part of microbial biomass (Perakis and Hedin, 2001).

To determine the ^{15}N enrichment in soil N fractions, sequential diffusion steps were performed for determining in the sequence NH_4^+–N, NO_3–N, and DON using the diffusion method (Brooks et al., 1989) with acidified filters (1 M oxalic acid, pH < 1) as described in detail by Wu et al. (2011). Diffusion steps were based on the transformation of all the respective soil N compounds to NH_4^+ and subsequent NH_3 volatilization by pH increase. The volatilized NH_3 was trapped on acidified filter disks used for analysis of ^{15}N enrichment at the Center of Stable Isotopes at Karlsruhe Institute of Technology (Garmisch-Partenkirchen, Germany) using a Flash EA 1112 Elemental Analyzer (Thermo Electron, Rodano, Italy) coupled to a Finnigan Delta Plus XP (Thermo Fisher Scientific, Bremen, Germany) ion ratio mass spectrometer (Dannenmann et al., 2009).

The ^{15}N enrichment in microbial biomass was calculated from additional release of ^{15}N in total dissolved N extracted from fumigated soil compared to unfumigated control soil (Wu et al., 2011).

Finally, ^{15}N excess enrichment for the target fractions was calculated by subtracting ^{15}N natural abundance.

2.7 Statistical analysis

Statistical analysis was done using the statistical computing environment R 3.0.1 (R Core Team, 2013). Besides computing mean values and standard errors for variables like general soil parameters and plant tissues, we fitted a two-way ANOVA model with interaction

$$Y_{ij,k} = \mu_0 + \alpha_i + \beta_j + \gamma_{ij} + e_{ij,k},$$

to the ^{15}N concentrations in bulk soil and SOM fractions. In the above model equation α_i, β_j and γ_{ij} represent the main effect of the factor treatment, the main effect of the factor time and the interaction effect between treatment and time, respectively. The factor time has four levels with independent observations, since samples were taken from different mesocosms, which can be regarded as mere repetitions. Differences between the four levels of the time factor within each treatment level were further tested by applying the Tukey test in a post-hoc test procedure.

3. Results

3.1 General soil characteristics

The Ah horizon was characterized by a slightly acidic pH, dominated by clay (Table 2). The bulk soil had an OC concentration of 60.7 mg g^{-1} and an N concentration of 4.7 mg g^{-1}. Because the analyses of the bulk soil samples revealed mean IC content < 1% of TC, we assumed the SOM fractions to be free of carbonates. The TC concentration therefore equaled the OC concentration. The bulk density was 0.79 g cm^{-3}. Mean soil temperature (5 cm depth) during the experiment differed between sites (Table 3): 10.3 °C (NW, control) and 11.1 °C (SW, drought). Soil water content in the control treatment was within the range of field capacity during the experimental period. The water content of the drought-treated soil mesocosms was smaller, limiting the plant available water content, especially during the roof period (Fig. 1). After 32 days, the coarse pores of the drought-treated soil mesocosms were completely filled with air. Some of the medium pores still contained some plant available water, consisting of adhesive water (Fig. 1; t3 *d* and t4 *d* > pF 2.5).

3.2 Masses, OC, and N in SOM fractions

The total mass recovery of the soil fractionation was 88 ± 1% for both treatments including unlabeled samples. The mass distribution of the different SOM fractions was uniform for both control and drought-treated soil mesocosms, with clay as the largest fraction. We detected no substantial differences between the treatments in the C and N concentrations of the SOM fractions (Table 4). The highest mean C and N concentrations were found in the light fractions (LF), with up to 456.3 mg C g^{-1} (oLF > 20 μm, control) and 14.6 mg N g^{-1} (oLF > 20 μm, drought), followed by the clay fraction (control), with up to 57.9 mg C g^{-1} and 6.9 mg N g^{-1}, respectively. Regarding the distribution of N within the bulk soil, the largest amounts of N were stored in the clay fraction (control) with up to 3.3 mg N g^{-1} soil, representing nearly 75% of bulk soil N. Six to 10% of the N from the clay fraction was exchangeable bound NH_4^+–N. The rest was present in organic forms. This distribution neither showed a temporal trend nor was it influenced by the different treatments. The C/N ratio of the LF was highest in the fLF (Table 4). The C/N ratio of the organo-mineral fractions showed the highest values in the sand-sized fraction and decreased with particle size to the lowest values in the clay fraction (8.0).

3.3 ^{15}N concentrations in bulk soil and SOM fractions

Directly after label application, the ^{15}N concentrations of bulk soil samples were slightly higher in control soil with 0.1464 atom% excess compared to drought soil with 0.1355 atom% excess, respectively, 7.46 mg ^{15}N excess kg^{-1} soil compared to 7.02 mg ^{15}N excess kg^{-1} soil (Fig. 3). The differences increased after 7 days with 0.1103 atom% excess (control) and 0.0868 atom% excess (drought), respectively, excess of 4.92 mg ^{15}N kg^{-1} soil compared to 3.78 mg ^{15}N kg^{-1} soil. The situation was reversed after 32 days, when the ^{15}N concentrations were lower in control soil (0.0420 atom% excess) compared to drought soil (0.0630 atom% excess). During this time, the climate change treatment effect was enhanced using a roof. A two-way ANOVA indicated a highly

Table 2
General parameters of the investigated soil from Ah horizon. Data is given as mean values of 5 replicates with standard errors for texture and pH. OC, IC, N, and C/N values are the mean of all 30 soil samples from this study, including standard errors.

Texture			OC	IC	N	C/N	pH
Sand [%]	Silt [%]	Clay [%]	mg g^{-1}	mg g^{-1}	mg g^{-1}		0.01 M $CaCl_2$
2000–63 μm	63–2 μm	<2 μm					
3.7 ± 0.6	28.5 ± 1.7	67.7 ± 2.2	60.7 ± 1.5	0.5 ± 0.2	4.7 ± 0.1	13	6.0 ± 0.2

OC = organic carbon.
IC = inorganic carbon.
N = nitrogen.

Table 3
Climatic parameters of the experimental sites (control on NW vs. drought on SW) during the experimental period (June–September 2011). The roof period was from 27 June 2011 to 9 August 2011. Horizontally installed combined soil moisture/temperature probes gathered data hourly in 5 cm depth (mean values of $n = 5$ measurements) in intact beech-soil mesocosms of the control and drought treatment each.

Treatment	Month/time period	Mean soil temperature °C	Volumetric soil moisture content $m^3\ m^{-3}$
Control	March	3.2	0.21
	April	8.0	0.22
	May	10.4	0.23
	June	12.7	0.26
	July	13.1	0.28
	August	15.0	0.28
	September	13.6	0.30
	October	9.2	0.31
	November	6.5	0.30
	Roof period	13.3	0.28
	Mean during experiment	10.3	0.26
Drought	March	5.2	0.23
	April	10.1	0.22
	May	11.2	0.22
	June	13.0	0.23
	July	13.4	0.18
	August	15.4	0.22
	September	14.1	0.25
	October	9.6	0.25
	November	6.8	0.24
	Roof period	13.6	0.18
	Mean during experiment	11.1	0.23

significant difference in ^{15}N concentration among bulk soils with respect to time (Fig. 3, Appendix S1 and S2). In case of atom% excess we also found a significant interaction between the time and the treatment effect.

Regarding the SOM fractions, we observed a general increase in ^{15}N in atom% excess for the fLF and the oLF > 20 μm for both treatments over time, whereas the values in the silt and clay fraction tended to decline in both treatments. The two-way ANOVA showed significant treatment effects for fLF and oLF > 20 μm (Figs. 3 and 4). The overall maximum in ^{15}N atom% excess was found in the drought-treated fLF after 32 days (Fig. 4). Aside from the first harvest, all drought-treated fractions (except clay) had higher ^{15}N atom% excess values than those of the control treatment (Fig. 4). ANOVA revealed treatment and time interaction effects in silt and clay fractions (Appendix S1 and S2). Time effects were observed in the control-treated silt fraction, whereas clay had time effects in both treatments.

We found the highest milligram concentrations of ^{15}N per kilogram of soil in the clay fraction at each sampling date (Fig. 5). Similar to the isotopic enrichment in atom% excess, the concentrations in milligrams per kilogram of soil tended to be higher in the drought SOM fractions than in the controlled ones.

The relative distribution of the label among the SOM fractions showed higher values in the LFs of the drought-treated soils (Table 5). The maximum with nearly 30% in the LFs was reached after 32 days, tripling the controlled LFs in their distribution. The tracer distribution declined in the clay fraction during the experiment (25% in drought and 16% in control after 97 days compared to 0 days). Except for the sand and silt fraction, the distribution changed markedly over time from day 0 to 97 d. Significant treatment effects appeared in the oLF > 20 μm ($p = 0.0317$) and in the clay fraction ($p = 0.0111$), where additionally significant time effects ($p = 0.0013$ for oLF > 20 μm and $p = 0.0000$ for clay) occurred within both treatments (Appendix S1 and S2). Significant time effects in the oLF > 20 μm occurred only in the drought treatment, and clay had further interaction effects between time and treatment ($p = 0.0073$).

3.4 N and ^{15}N content in soil microbial biomass, NO_3^-, NH_4^+, and DON

NO_3^- was the dominant form of extractable inorganic N during the first two sampling dates (Table 6) with up to 13.92 mg

Table 4
Mass distribution, carbon (C) and nitrogen (N) concentrations of soil fractions, and contribution of N from specific fractions to the bulk soil in control and drought-treated soils. Data is given as mean values with standard errors for 12 replicates pooled across all sampling dates (three replicates on four sampling dates).

Fraction	Control						Drought					
	Mass	C	N			C/N	Mass	C	N			C/N
	mg (g soil)$^{-1}$	mg (g fraction)$^{-1}$	mg (g fraction)$^{-1}$	mg (g soil)$^{-1}$	% of recovered bulk soil		mg (g soil)$^{-1}$	mg (g fraction)$^{-1}$	mg (g fraction)$^{-1}$	mg (g soil)$^{-1}$	% of recovered bulk soil	
fLF	8.2 ± 1.1	436.5 ± 5.0	12.2 ± 0.0	0.1	2.3	36.2	7.5 ± 0.6	444.8 ± 5.9	12.3 ± 0.4	0.1	2.2	36.3
oLF > 20 μm	6.9 ± 0.6	456.3 ± 4.2	14.5 ± 0.6	0.1	2.3	32.1	8.2 ± 0.9	451.2 ± 6.0	14.6 ± 0.5	0.1	2.8	31.4
oLF < 20 μm	22.2 ± 2.7	339.8 ± 5.7	14.6 ± 0.4	0.3	7.3	23.5	23.2 ± 2.1	318.2 ± 13.0	14.0 ± 0.6	0.3	7.7	22.9
Sand	107.0 ± 6.7	10.9 ± 2.3	0.4 ± 0.0	0.0	0.9	34.3	114.0 ± 9.2	9.8 ± 2.3	0.5 ± 0.1	0.1	1.3	20.9
Silt	257.5 ± 3.8	36.5 ± 3.0	2.1 ± 0.1	0.5	12.4	17.3	261.1 ± 3.0	30.2 ± 2.5	1.9 ± 0.1	0.5	11.7	15.8
Clay	481.7 ± 13.7	57.9 ± 2.1	6.9 ± 0.2	3.3	74.9	8.4	472.1 ± 12.3	53.3 ± 2.1	6.7 ± 0.2	3.1	74.3	8.0
Total	883.4			4.4	100.0		886.0			4.2	100.0	

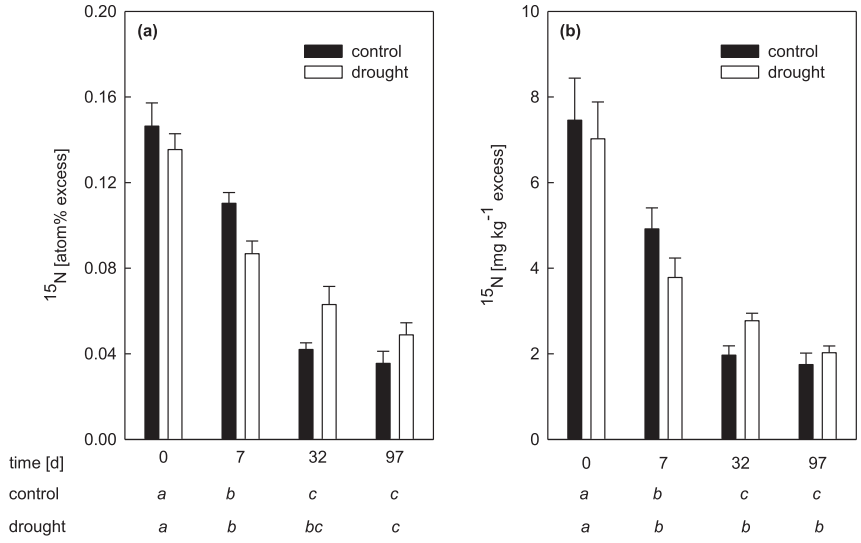

Fig. 3. ^{15}N concentration (a) in ^{15}N atom% excess and (b) milligrams per kilogram in the bulk soil. Data indicates mean of three replicates with standard errors. Italic letters indicate significant mean differences in time within each treatment group ($p < 0.05$).

N kg^{-1} sdw. ^{15}N excess concentrations in the inorganic extractable fractions declined during the experiment independent of the treatment. This trend was also present in the organic fractions with some variations. During the experimental period, N and ^{15}N concentrations of NO_3^-, NH_4^+, and DON were not considerably affected by summer drought. Seasonal fluctuations instead influenced the variation in the data. The maximum in the ^{15}N concentration of the MB-N was detected after 7 days in the control soil and after 32 days in the drought treatment.

3.5 N and ^{15}N in plant tissues

Our analysis of plant tissues showed a general decrease in N content during the growing season for the control plants (Appendix S3) from spring to summer. The N content of the stem declined from 12.2 to 9.2 mg per plant, that of the roots from 27.8 to 20.6 mg per plant, and that of the leaves from 25.6 to 12.6 mg per plant. The ^{15}N content of the plant tissues increased with time consistently in both treatments (Appendix S3). After only a few hours, the ^{15}N label could be detected in the roots of both treatments. After 97 days, the ^{15}N excess concentrations of the leaves in the controlled systems were twice as high as the values from the drought-treated plants (Appendix S4).

3.6 Recovery of ^{15}N label

Higher overall recoveries occurred in the drought treatment compared to the control for the last two samplings (Table 7). The treatments were not significantly different. The recovery in MB-N, NH_4^+, NO_3^-, and DON decreased significantly with time. In contrast, the non-extractable soil fractions decreased in their recoveries during the first 7 days and increased thereafter, stabilizing at 25% (drought) and nearly 22% (control), respectively. At 0 and 7 days, most of the recovered label was present as MB-N, NH_4^+, NO_3^-, and DON. After 32 days, most of the recovered ^{15}N was stabilized in non-extractable soil fractions. Generally, the recovery in the plant tissues

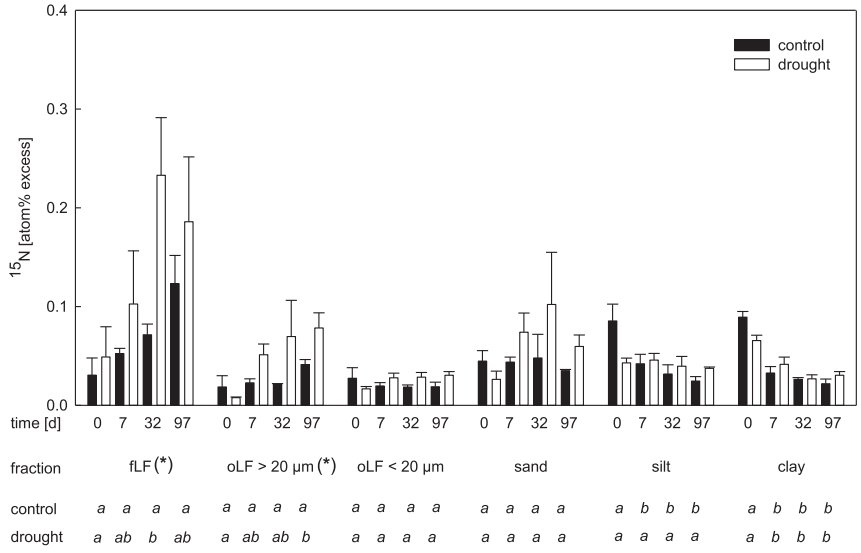

Fig. 4. ^{15}N concentration in ^{15}N atom% excess in soil organic matter (SOM) fractions (fLF = free light fraction, oLF = occluded light fraction). Data indicates mean of three replicates with standard errors. Asterisks in brackets after the fraction ($^*p < 0.05$) indicate significant differences between the treatments for this fraction over the experimental period (0, 7, 32, 97 days). Italic letters indicate significant mean differences in time within each treatment group ($p < 0.05$).

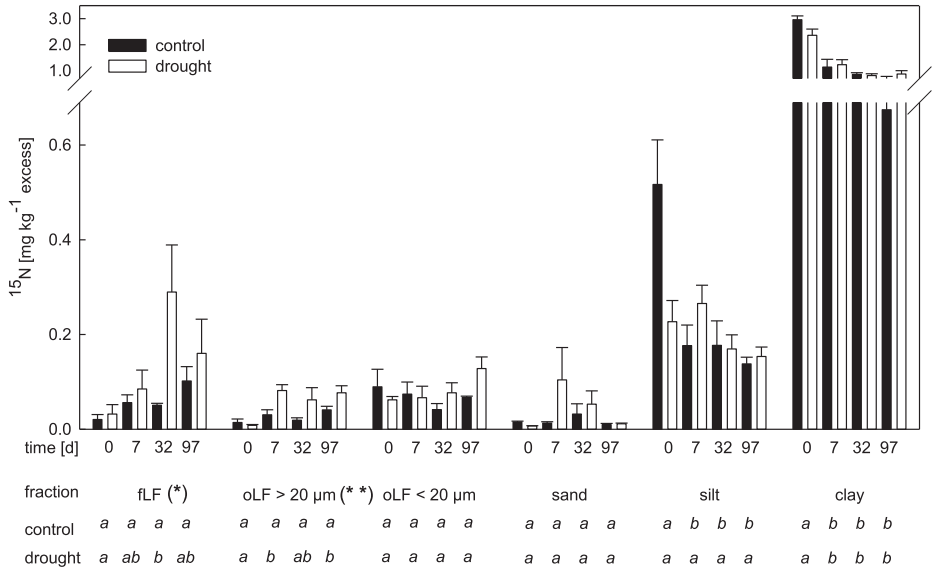

Fig. 5. ^{15}N excess of soil organic matter (SOM) fractions in milligrams per kilogram of soil (fLF = free light fraction, oLF = occluded light fraction). Data indicates mean of three replicates with standard errors. Asterisks in brackets after the fraction ($^{*}p < 0.05$; $^{**}p < 0.01$) indicate significant differences between the treatments for this fraction over the experimental period (0, 7, 32, 97 days). Italic letters indicate significant mean differences in time within each treatment group ($p < 0.05$).

Table 5
Mean percent distribution of recovered ^{15}N label among soil organic matter (SOM) fractions (fLF = free light fraction, oLF = occluded light fraction).

Treatment	Days	Distribution [%]						Total
		fLF	oLF > 20	oLF < 20	Sand	Silt	Clay	
Control	0	0.6 ± 0.9	0.4 ± 0.0	2.5 ± 0.2	0.4 ± 0.0	14.3 ± 0.8	81.8 ± 0.1	100
	7	3.8 ± 3.0	2.0 ± 0.4	5.0 ± 1.5	0.9 ± 2.8	11.8 ± 0.8	76.5 ± 3.0	100
	32	4.3 ± 6.9	1.6 ± 1.8	3.5 ± 1.4	2.7 ± 2.0	15.0 ± 1.9	72.8 ± 4.5	100
	97	9.9 ± 4.9	3.9 ± 0.6	6.5 ± 0.9	1.0 ± 0.1	13.4 ± 2.4	65.4 ± 3.2	100
Drought	0	1.2 ± 0.3	0.3 ± 0.2	2.3 ± 1.1	0.3 ± 0.0	8.4 ± 2.4	87.5 ± 2.1	100
	7	4.6 ± 1.2	4.5 ± 0.8	3.6 ± 1.7	5.7 ± 0.3	14.5 ± 2.8	67.2 ± 2.2	100
	32	19.8 ± 0.8	4.2 ± 0.6	5.3 ± 1.4	3.6 ± 1.5	11.6 ± 2.7	55.5 ± 2.5	100
	97	11.4 ± 2.8	5.5 ± 1.3	9.1 ± 1.0	0.8 ± 0.2	11.0 ± 0.3	62.2 ± 3.8	100

increased during the experimental period, with higher values in the drought treatment, but remained very low compared with the other fractions. ANOVA revealed treatment and time interactions for extractable soil fractions and additionally a time effect for extractable soil fractions and plant tissues (Appendix S1 and S2).

4. Discussion

4.1 Partitioning of N and ^{15}N between bulk soil and SOM fractions

The results revealed that bulk soil N and the partitioning of total N between the different SOM fractions were similar for both treatments, showing that the transfer treatment did not affect N pool size. Nearly 75% of total N was found in the clay fractions of both treatments (Table 4), showing the importance of organo-mineral associations for N stabilization. Overall, an enrichment of N is observed with decreasing particle size, resulting in very low C/N ratios in the fine organo-mineral fractions (Table 4; Stemmer et al., 1998; Kölbl et al., 2006; Bimüller et al., 2013). We estimate that < 10% of total N is fixed as NH_4^+ in illite (i.e. in mineral interlayers), which cannot be exchanged (Nieder et al., 2011).

Conspicuous effects in ^{15}N partitioning, however, were already apparent after one single summer drought during the experimental period. The incorporation of the added inorganic ^{15}N label into the

Table 6
Concentrations of total N and ^{15}N excess in ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic nitrogen (DON), and microbial biomass (MB) fractions after 0, 7, 32, and 97 days after label application. Data indicates means of three replicates with standard errors.

Treatment	Days	N [mg (kg soil)$^{-1}$]				^{15}N [mg (kg soil)$^{-1}$] excess			
		NH_4^+	NO_3^-	DON	MB-N	NH_4^+	NO_3^-	DON	MB-N
Control	0	3.86 ± 1.69	13.92 ± 2.85	2.82 ± 1.99	54.50 ± 1.96	0.70 ± 0.30	3.13 ± 0.16	0.09 ± 0.07	0.69 ± 0.13
	7	7.80 ± 1.86	12.12 ± 0.82	4.90 ± 2.79	88.67 ± 11.38	0.52 ± 0.17	2.48 ± 0.04	0.10 ± 0.05	1.11 ± 0.42
	32	3.64 ± 1.02	5.17 ± 2.05	5.14 ± 1.00	106.60 ± 23.34	0.05 ± 0.03	0.32 ± 0.14	0.03 ± 0.01	0.36 ± 0.14
	94	4.04 ± 0.48	0.49 ± 0.18	6.00 ± 0.55	84.51 ± 10.56	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.22 ± 0.05
Drought	0	6.45 ± 1.47	10.78 ± 2.26	1.84 ± 2.51	47.39 ± 0.93	1.13 ± 0.32	2.94 ± 0.44	0.04 ± 0.06	0.46 ± 0.33
	7	5.05 ± 0.98	5.62 ± 0.58	5.32 ± 1.58	88.35 ± 5.42	0.16 ± 0.04	1.60 ± 0.20	0.09 ± 0.02	0.33 ± 0.06
	32	2.50 ± 0.50	2.42 ± 2.11	6.12 ± 0.98	110.04 ± 28.76	0.03 ± 0.00	0.32 ± 0.30	0.04 ± 0.00	0.62 ± 0.22
	94	3.19 ± 0.35	0.40 ± 0.09	7.01 ± 0.98	70.14 ± 2.10	0.02 ± 0.00	0.01 ± 0.00	0.03 ± 0.01	0.22 ± 0.02

Table 7
Percent recoveries of added label in extractable soil, non-extractable soil, and plant tissues after 0, 7, 32, and 97 days after label application. Extractable soil is the sum of soil microbial biomass (MB-N), ammonium (NH_4^+), nitrate (NO_3^-), and dissolved organic matter DON. Non-extractable soil was calculated from the recoveries of the bulk soil minus the recoveries of the extractable soil fractions with the latter including chloroform-labile microbial biomass. Data indicates means of three replicates with standard errors.

Treatment	Days	Recovery [% of added label]			
		Extractable soil (MB-N, NH_4^+, NO_3^-, and DON)	Non-extractable soil	Plant tissues	Total
Control	0	67.10 ± 6.40	41.46 ± 11.94	0.00 ± 0.00	108.56
	7	61.18 ± 7.78	10.41 ± 2.00	0.03 ± 0.01	71.62
	32	11.11 ± 3.74	17.54 ± 2.57	0.04 ± 0.01	28.69
	97	3.91 ± 0.82	21.53 ± 3.14	0.05 ± 0.01	25.49
Drought	0	66.60 ± 7.96	35.61 ± 16.53	0.00 ± 0.00	102.22
	7	31.58 ± 1.80	23.51 ± 4.82	0.03 ± 0.01	55.11
	32	14.71 ± 7.50	25.66 ± 5.14	0.06 ± 0.01	40.43
	97	4.15 ± 0.29	25.34 ± 2.08	0.08 ± 0.01	29.57

SOM fractions occurred very quickly during our experiment in both treatments. Similar to the results of Compton and Boone (2002), we detected a faster overall cycling of NH_4^+, because less ^{15}N–NH_4^+ was detected during all samplings compared to ^{15}N–NO_3^- (Table 6). A few hours after label application, one third of the ^{15}N label was already recovered in the SOM fractions (Table 7). From day 7 onwards, the LFs were enriched, revealing that these fractions include an important part of the active-phase SOM, as described by Balabane and Balesdent (1992). The applied ^{15}N label was particularly associated with the clay fraction immediately after application (>80%, Table 5). The distribution of the ^{15}N showed higher values in the clay (time 0, Table 5) compared to the distribution of total N (Table 4), which demonstrates that the recently applied ^{15}N was preferentially found there compared to all other fractions. The present mineralogy of the clay fraction supports this, because illite temporarily stores $^{15}NH_4^+$. The fixation after fertilizer dressing usually occurs within hours (Nieder et al., 2011). Due to significantly declining absolute concentrations (Fig. 5) in the clay fractions throughout the experiment, we assume that to a large extent the immediately attached $^{15}NH_4^+$ was not sustainably stabilized in these fractions. Balabane (1996) observed an exponential decrease of labeled ^{15}N in the clay fraction over 3 years with a rapid decline during the first 12 months because labeled $^{15}NH_4^+$ was not firmly attached. In a field experiment, Kowalenko (1989) detected a direct fixation of NH_4^+ by clay but a strong decrease after 14 days and a small but constant portion throughout the remainder of the ^{15}N. The rapidly and tightly fixed NH_4^+ from fertilizer application is slowly released again with a decrease in NH_4^+ concentration in the soil solution, a process that is supported by heterotrophic microorganisms (Nieder et al., 2011). Recous et al. (1988) also reported that $^{15}NH_4^+$ fixation was not involved to a significant extent in the amount of labeled N measured as organic ^{15}N in their studies. We assume that the majority of $^{15}NH_4^+$ in the clay fraction was net compensated by organic ^{15}N and microbial residues during later stages of the experiment. The applied inorganic $^{15}NO_3^-$ and especially $^{15}NH_4^+$ are preferentially immobilized and are therefore expected to be quickly re-utilized for biological production and thus converted into organic N structures. These microbial products from originally labile organic N become stabilized with the mineral soil matrix in organo-mineral associations (Cotrufo et al., 2013). This is expected to be a major N protection pathway because N is sequestered from the overall N cycle and becomes stabilized by incorporation into mineral-associated SOM, where it resists further attack by microorganisms and is not readily available to plants.

4.2 Drought effects enhanced ^{15}N concentrations in the light fractions

In contrast to the amounts of total soil N, the distribution of ^{15}N excess among the different SOM fractions clearly showed differences between the two treatments at the end of the experiment. The summer drought had a significant effect on the ^{15}N concentrations in the fLF and oLF > 20 μm. We ascribe the increasing relative distribution in the drought-treated LFs (Table 5) to the microbial biomass associated with the LFs functioning as a reservoir of readily available nutrients (Kanazawa and Filip, 1986). This increasing trend in the LFs led, in turn, to a decreasing proportional distribution in the clay fraction (Table 5).

Drought generally works as a suppressor of N cycling (Andresen et al., 2010), including the investigated soil system (Dannenmann et al., 2009). The drought-treated plant–soil–microbe system was less active than the control system, because the MIT was delayed (M. Dannenmann, personal communication), thus retarding the transfer into the mineral associations. Most of the applied label was found in the MB-N of the drought-treated systems after just 32 days, whereas the control showed an even higher maximum after only 7 days (Table 6). All processes seemed to be prolonged, but they were not stunted, which means the same flux occurred under drought treatment over a longer period of time. This is in line with the results of Garten et al. (2009), who showed that reduced soil moisture slowed soil C cycling. Kuster et al. (2013) found a strongly inhibited microbial activity reduced by drought similar to our study. Microbial activity is highest at about pF 2 and decreases as soil becomes drier (approximately pF 4.7–5) or saturated (approximately pF 0) (Nadelhoffer et al., 1995). The soil microorganisms were associated with the LF, but they were less active in the drought-treated soil systems. Nevertheless, they reached the N source more easily than the plants in our experimental assembly, because they could cope better with the drier conditions.

Compton and Boone (2002) reported that drought effects decreased the immobilization of inorganic N. Several studies indicated that drying soil reduced the microbial activity and a subsequent rewetting process revived it (e.g. Bottner, 1985; Van Gestel et al., 1992; Schimel et al, 2007). The activity of soil microbes is not only related to soil moisture content but also to the accessibility of nutrients and SOM, among other factors. The wetting flush may be ascribed to both a reconstituting mineralization of SOM and the mineralization of previously unavailable, easily decomposable organic substrates, especially microbes killed during drying and rewetting (Wu and Brookes, 2005; Borken and Matzner, 2009). This latter flush caused by a priming effect has been termed the Birch effect (Jarvis et al., 2007). It was not included in our experiment because we sampled for only 48 days after the removal of the roof, when a direct flush was no longer recognizable. However, we recovered more label in the drought-treated plant–soil mesocosms after 97 days (Table 5).

4.3 Lower ^{15}N stabilization in organo-mineral associations due to desiccation

Our findings concerning the key N cycling processes revealed that wetter soils have shorter stabilization periods, while drier soils take longer to stabilize N but have greater potential for N loss before stabilization in organo-mineral associations follows. Stabilization of recent N inputs occurs mainly in silt and especially clay fractions (Balabane and Balesdent, 1995; Bimüller et al., 2013). Our results verified the hypothesis that significantly less label was stored in the organo-mineral associations of the clay fraction (Table 4) due to the drought treatment. The proportional distribution was shifted in favor of a higher amount of label being stored in

the LFs. First, water, the transport agent, was missing in the drought-treated soil systems (Fig. 1), but it was available to transport the label in the control soil mesocosms. Second, we ascribed the higher label concentrations in the LFs to less active microbes in the drought treatment compared to control. The microbial turnover is reduced by desiccation, because smaller amounts of ^{15}N can be assimilated by soil microorganisms, converted into their organic structures, and consequently stabilized by adsorption on mineral surfaces. This emphasizes the important role of soil microorganisms in N stabilization, although we did not detect a significant treatment effect (Table 6). The storage in the labile pools of the drought soils over longer periods may increase the risk of substantial leaching and erosion under heavy rainfall events. Experiencing an extreme rainfall event after several consecutive summer droughts may therefore lead to less N stabilization. Several studies (Sparling and Ross, 1988; Van Gestel et al., 1993a, b) reported a reduced microbial biomass under drought stress due to higher soil moisture tension. Our results from one summer drought showed effects at the beginning of the stabilization chain of inorganic ^{15}N in the labile fractions. When transferring these effects to climate change scenarios, we assume that N stored in microbial biomass may be mineralized during the Birch effect due to rewetting events in autumn. The plants may not incorporate N during the fast mineralization flush and the risk of N loss due to leaching or erosion under heavy rainfall events may increase (Borken and Matzner, 2009; Matías et al., 2011). We therefore assume that prolonged summer droughts may alter the N distribution pattern.

4.4 Drought retards stabilization dynamics

With respect to expected prolonged summer droughts in Central Europe leading to lower soil water availability, we presume from the results presented here that N stabilization processes may be inhibited by desiccation. Microbial biomass activity strongly depends on water availability, with retarded MIT under drought conditions. These findings during only one growing season showed that recently applied inorganic N responded with higher ^{15}N concentrations in the LFs under drought conditions. When microorganisms are less active under drought conditions, all N cycle processes slow down. The extended residence time in the labile fraction increases the risk of leaching losses under extreme rainfall events, which are predicted by Intergovernmental Panel on Climate Change IPCC (2007) scenarios for Central Europe. The retarded stabilization rate suggested by our findings may lead to an altered N distribution pattern. The modified tracer partitioning in different SOM fractions, as revealed by our experiment, may serve as a central component in models of future ecosystem responses.

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Appendix A. Supplementary data

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Study III

Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil

by

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Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil

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ABSTRACT

To better understand how carbon and nitrogen mineralization are linked in soils, we conducted a long-term incubation experiment and compared carbon and nitrogen dynamics in the bulk soil and in soil fractions. Topsoil of a Rendzic Leptosol from a beech forest site near Tuttlingen, Germany, was separated into three particle size classes: sand (2000–20 μm), silt (20–2 μm), and clay (<2 μm). Bulk soil and particle size fractions were incubated in replicate, allowing periodic destructive sampling of triplicates at day 0, 14, 42, 84, 140, 210, and 280. We monitored CO_2–C respiration, NH_3–N emissions, nitrogen mineralization, pool sizes of total and salt extractable (0.01 M $CaCl_2$) organic carbon and nitrogen, and microbial biomass carbon and nitrogen. The chemical composition of selected samples was further characterized by ^{13}C-NMR spectroscopy. Fractionation did not influence carbon mineralization ($\sum$ fractions ≈ bulk soil), which decreased in the order sand > clay > silt. The fractions respired between 10.4% (sand fraction), 8.8% (clay fraction) and 4.4% (silt fraction) of total soil organic carbon. However, nitrogen mineralization was affected by the fractionation procedure ($\sum$ fractions < bulk soil) and followed the order clay > silt > sand. Fractionation increased the surface area and hence provided accessory mineral surfaces, which allowed new binding of especially nitrogen-rich compounds, in addition to ammonium fixation via cation exchange. As indicated by lower metabolic quotients, microbial carbon mineralization was more efficient in the bulk soil compared to the calculated sum of fractions. In the clay fraction, carbon mineralization rates, salt extractable organic carbon contents, and microbial biomass carbon and nitrogen contents declined strongly towards the end of the incubation. This indicates that in the clay fraction, organic carbon was not available for microbial degradation and that microorganisms were strongly carbon-limited causing a subsequent inhibition of nitrogen immobilization. Density fractionation revealed that organic matter in the sand fraction consisted mainly of particulate organic matter present as light material containing partly decomposed plant remnants. The organic matter in the clay fraction was mostly adsorbed on mineral surfaces. Organic matter in the sand and in the clay fraction was dominated by O/N-alkyl C indicating low recalcitrance, but the C/N ratio of organic matter narrowed with decreasing particle size. Our results suggest that carbon and nitrogen mineralization are decoupled in the mineral-associated fractions of the soil. The specific interactions of both carbon and nitrogen containing components with the mineral matrix strongly modulate the mineralization dynamics. Therefore, isolated considerations of C/N or alkyl C to O/N-alkyl C ratios of organic matter are insufficient as indicators for decomposition in plant residues. The combined consideration of C/N and alkyl C to O/N-alkyl C ratios provides a first impression about the degree of decomposition in plant residues. However, bioavailability in fractions where organic matter is mainly stabilized by spatial inaccessibility and by organo-mineral interactions cannot be explained by these ratios, but can be examined by an incubation approach.

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0038-0717/

1. Introduction

Soil organic matter (SOM) can be subdivided in three functional pools that are stabilized by specific mechanisms, leading to specific turnover times (Collins et al., 2000; von Lützow et al., 2007): (1) the active pool consisting of microbial biomass and metabolites (turnover <10 years); (2) the intermediate pool built up by partially stabilized organic matter (turnover 10–100 years); and (3) the passive pool (turnover >100 years) (von Lützow et al., 2008). Three stabilization mechanisms protect SOM from decomposition: (1) selective preservation due to recalcitrance; (2) protection by occlusion; and (3) interaction with minerals and metal ions (Sollins et al., 1996; von Lützow et al., 2006). Selective preservation of recalcitrant compounds is relevant for the active pool but does not explain long-term stabilization. Occlusion of SOM by biogenic aggregation operates in the intermediate pool while occlusion of SOM in clay microstructures by abiotic aggregation and the formation of organo-mineral interactions were found to be important mechanisms in the passive pool (von Lützow et al., 2008).

Assuming that sorption is a major stabilization mechanism, SOM within the sand fraction is assigned to the active pool, and SOM in silt and clay fractions to the intermediate and passive pool (von Lützow et al., 2007). Although fractionation procedures can create slight redistributions of SOM (Cambardella and Elliott, 1994; Gregorich et al., 2006), the main advantage is the analytical characterization and comparison of individual soil fractions and their comparison with and contribution to the bulk soil, which is otherwise not accessible. Studying decomposition and biodegradability, such as mineralization, in the different particle size fractions allows evaluation of the stability of SOM associated with these fractions (Christensen, 1987). Therefore, particle size separation with subsequent incubation experiments provides a useful approach to describe the SOM stability within discrete fractions obtained from a complex matrix (Crow et al., 2007).

Previous researchers have proposed mechanisms of SOM stabilization based on spatial orientation and interactions with the organo-mineral phase (Ellerbrock et al., 2005; Kleber et al., 2007), but there have been few attempts to demonstrate this by incubation experiments focusing on both carbon and nitrogen mineralization, despite the fact that carbon and nitrogen dynamics are closely associated (McGill et al., 1975; Gärdenäs et al., 2011). Few studies have investigated either carbon or nitrogen in SOM fractions (Sollins et al., 1984; Christensen, 1987; Swanston et al., 2002; Mueller et al., 2014), but, instead, investigations combining carbon and nitrogen dynamics have been mainly carried out on bulk soil (Robertson et al., 1988; Joergensen et al., 1990; Raubuch and Joergensen, 2002; Weintraub and Schimel, 2003). The simultaneous evaluation of carbon and nitrogen dynamics in individual SOM fractions has been achieved only in two studies until present. Swanston et al. (2004) applied this approach in a nitrogen fertilization experiment. They found higher carbon and nitrogen mineralizations from loamy and sandy bulk soils compared to those obtained from recombined and incubated density fractions. Relationships between carbon and nitrogen mineralization were negative in the light fraction with particulate organic matter (POM), indicating net nitrogen immobilization, and were positive in the heavy fraction with mainly mineral-associated SOM. They identified the latter, more stable fractions as sources for nitrogen release. Parfitt and Salt (2001) incubated particle size fractions in an agricultural loamy soil and considered the clay fraction as a major site of nitrogen mineralization. They found that the carbon mineralization in soil fractions was related to the availability of substrates rather than to the C/N ratio, because SOM in the different particle size fractions behaved differently according to its specific decomposition status. However, a detailed understanding of the processes that impact turnover of carbon and nitrogen is still missing. Specifically it is not clear how the stabilization of SOM in the fine soil fractions affects the interactive mineralization of C and N. Thus, in this study we aimed to achieve a more complete understanding of the control of carbon and nitrogen turnover with respect to its different SOM pools by analyzing net mineralizable carbon and nitrogen pools, microbial biomass carbon and nitrogen, and total carbon and nitrogen in specific soil fractions.

We separated a clay-rich topsoil of a Rendzic Leptosol from a beech forest site near Tuttlingen, Germany, into particle size classes at cut-offs of 20 μm and 2 μm, and we termed the fractions sand (2000–20 μm), silt (20–2 μm), and clay (<2 μm). We used extended laboratory incubation (280 d) of bulk soil and the sand, silt, and clay fractions to analyze the SOM in physical soil fractions. We measured mineralized nitrogen pools (N_{min}), microbial biomass carbon and nitrogen, and their CO_2–C and NH_3–N release at several time points during incubation. Essential information on the composition of SOM in the different fractions is obtained from solid-state ^{13}C-NMR spectroscopy. Our study includes data on microbial biomass showing the efficiency and limitations of the microorganisms during mineralization. These data are vital to understanding the mineralization behavior of the different SOM pools. Differences between the artificial fractions and the bulk soil illustrate the relative importance and function of the fractions in the soil as a whole at this site. This approach allows quantifying the potential bioavailability of carbon and nitrogen in the different particle size fractions in order to improve our understanding of soil carbon and nitrogen stabilization patterns in specific SOM fractions and to develop predictive parameters for carbon and nitrogen cycling.

2. Materials and methods

2.1. Site characteristics and collection and storage of soil material

On October 16, 2010, 10 kg of soil were sampled randomly from three spots on the northwest slope at the experimental site in a beech dominated forest near Tuttlingen (Swabian Jura, Baden-Württemberg, Germany, 47°59′N, 8°45′E), derived from the upper 15-cm horizon of a Rendzic Leptosol after removing the O-layer. The site was selected because it is well characterized concerning the ecosystematic N dynamics (Dannenmann et al., 2006, 2007, 2009; Bimüller et al., 2014). Furthermore, this mull site is known to have a high aggregation with a slow aggregate turnover (Bimüller et al., 2013). The studied Ah horizon material had a texture classified as heavy clay and a pH of 6 (FAO, 2006; Bimüller et al., 2014). The material was composited and stored at 4 °C in tightly sealed bags until sieving with a mesh size of 2 mm. Bulk soil had a mean organic carbon concentration of 64.8 mg g^{-1} and a mean nitrogen concentration of 4.6 mg g^{-1} (Table 1).

Bulk mineralogy by X-ray diffraction showed a relative enrichment of the clay fractions in phyllosilicates, especially irregular mixed-layer illite-smectite and kaolinite. Quartz dominates in the larger grain sizes (Bimüller et al., 2013).

2.2. Physical fractionation

To isolate SOM fractions for the incubation experiment, a particle size fractionation scheme (Appendix S1) was used for the subsequent decomposition study rather than density fractionation (Magid et al., 1996), thus avoiding the latter's repressive effects on microbial activity. Air-dried soil was dry-sieved by hand to <2 mm. A two-step ultrasonic disruption (Amelung and Zech, 1999) was performed according to Mueller et al. (2012), breaking up macro-

Table 1
Mean values of mass contribution, organic carbon concentration, inorganic carbon concentration, and OC/N ratios of soil fractions at the beginning of incubation (after they were equilibrated for 14 d to a soil water potential of 300 hPa). Carbon and nitrogen concentrations are calculated from three replicates, and standard errors are given here. Different lowercase letters indicate significant differences between measured substrates (α-level = 0.05).

Substrate	Mass	OC		IC		N_{tot}		OC/N	
	mg g^{-1}	mg g^{-1}		mg g^{-1}		mg g^{-1}			
Bulk soil		64.8 ± 0.9	a	0.9 ± 0.0	a	4.6 ± 0.1	a	14.1 ± 0.1	a
Sand	132.2	91.0 ± 6.6	b	10.5 ± 2.4	b	2.9 ± 0.2	b	30.9 ± 0.5	b
Silt	295.2	66.5 ± 0.6	a	0.2 ± 0.0	a	3.2 ± 0.0	b	21.1 ± 0.1	c
Clay	572.5	55.1 ± 0.1	a	0.7 ± 0.1	a	5.4 ± 0.0	c	10.3 ± 0.0	d

OC: organic carbon, IC: inorganic carbon, N_{tot}: total nitrogen.

aggregates with the first step (60 J ml^{-1}) and destroying microaggregates during the second step (450 J ml^{-1}). Coarse and medium sand fractions, containing fresh POM, were separated after the first ultrasonication step with a sieve of 200 μm mesh size. Fine sand and coarse silt fractions were separated after the second ultrasonication step, using a sieve of 20 μm mesh size. Fractions >20 μm were subsequently recombined according to their percentage of weight in the bulk soil. Medium and fine silt were jointly separated from the clay fraction (<2 μm) by sedimentation using Atterberg cylinders at 20 °C. In the following, the fractions are called sand (2000–20 μm), silt (20–2 μm), and clay (<2 μm). Coarse silt and sand fractions were combined, because their chemical compositions are similar and they accounted for minor proportions of the weight.

After fractionation, the obtained material was freeze-dried under vacuum at 20 °C according to standard protocols (Christensen, 1992) in order to yield homogeneous fractions and to avoid re-aggregation during air-drying. The fractionated material was stored at room temperature until the incubation experiment began. The bulk soil was only air-dried, because we wanted to conserve the natural soil structure. We applied a subsequent density fractionation using sodium polytungstate (1.7 g cm^{-3}, Appendix S1) to aliquots of the incubated substrates (n = 3) to evaluate the content as well as the chemical composition of the non-mineral-associated light fraction (LF) versus mineral-associated SOM (MIN).

2.3. Experimental design of the incubation procedure

To minimize anaerobic conditions and caking during incubation by simulating a natural soil structure (Swanston et al., 2002; Crow et al., 2007; Creamer et al., 2013), fifteen grams (dry weight) of each fraction was mixed thoroughly with annealed quartz sand (Quartz Sand Haltern, H33, average grain size 0.27 mm, Quarzwerke GmbH, Frechen, Germany) in a mass ratio of 1:1. We assume that the quartz sand was inert. Initial soil organic carbon (SOC), total nitrogen (N_{tot}), ammonium (NH_4^+), nitrate (NO_3^-), salt-extractable organic carbon (SEOC) and nitrogen (SEON), and microbial biomass were measured for the pure added quartz sand (Appendix 2). These values for the quartz sand were considered in the calculation of all sample values.

The homogeneous substrate mixtures were poured into incubation vessels (height 50 mm) that were prepared from plastic pipes with an inner diameter of 36 mm. To ensure good air diffusion, all vessels were prepared with eight holes (diameter 7 mm) with 90° offset, adopted from Mueller et al. (2012). To prevent material losses through these holes, we lined all vessels filled with bulk soil, sand, and silt with an inert nylon fabric. The vessels for the clay fraction were prepared with a fine-mesh polyamide membrane (0.45 μm, ecoTech, Bonn, Germany) to avoid material losses, whereas for the coarser sample materials, the nylon cloths were sufficient. Both fabrics lining the vessels were chosen with respect to the specific pore systems of the incubated fractions or bulk soil and sustained sufficient air diffusion. The bottom of each vessel was prepared with a water-permeable micro-glass fiber paper with a diameter of 90 mm (MG 160, Munktell, Bärenstein, Germany) fixed with a steel wire. All substrate treatments were carried out in triplicate.

All soil-filled vessels were saturated with distilled water in a water bath and were allowed to equilibrate for 24 h, so the pores of the substrates were completely filled with water. Over the following 14 d, samples were equilibrated to a soil water potential of 300 hPa (corresponding to water-holding capacity with 45 %vol) on a porous fired clay plate coupled to a pressure extractor (pF Laborstation 4251 and pF Druckstufenmodul 4252, EcoTech, Bonn, Germany) in the dark at 25 °C. This pre-incubation removed the first flush of carbon mineralization caused by rewetting (Franzluebbers et al., 1996). We chose the field capacity to adjust the samples to an ecologically meaningful soil water potential, because this is an accurate index of water availability to microorganisms (Harris, 1981). The activity of aerobic microbes is greatly dependent on soil water availability, which governs the transport of oxygen and substrates (Young and Ritz, 2000).

Before starting incubation, the substrate mixture was homogenously inoculated by pipetting 100 μl of inoculum solution prepared from the corresponding fresh intact bulk soil (stored at 4 °C and transferred to room temperature overnight before use). This solution was obtained by shaking soil material from the experimental site in distilled, sterile water for 3 h (1:10 soil:water) (Crow et al., 2007). The inoculation provided the same capability for microorganisms in each substrate after a partial kill-off (Christensen, 2001) due to the impact of ultrasonication and freeze-drying during fractionation.

During the incubation period of 280 d, each vessel with a substrate sample was incubated in an air-tight 0.9 L jar and stored in a dark room at a constant temperature of 20 °C. Destructive sampling of three vessels per substrate treatment (n = 3) was performed after 0, 14, 42, 84, 140, 210, and 280 d. The acidity of incubated substrates mixed with quartz sand was measured in duplicate from the substrates of the first sampling using a pH meter (WTW pH 340, Weilheim, Germany) in 0.01 M $CaCl_2$ at a ratio of 1:2.5 (soil:solution) with a glass electrode (Hamilton, Höchst, Germany). Soil moisture contents were determined gravimetrically after placing subsamples in a drying oven at 105 °C for 24 h. We checked and preserved the soil water conditions throughout the incubation period to guarantee an unbiased estimate of turnover rates and pool sizes (Yoo et al., 2006). Therefore, soil moisture contents were monitored with every destructive sampling and randomly between samplings. We readjusted soil moisture contents for the first time after 105 d by pipetting 1 ml of distilled water into each of the remaining soil vessels and for the second time after 231 d. The jars were vented every week to prevent toxic CO_2 accumulations and to return to ambient CO_2 concentration.

2.4. Nuclear magnetic resonance spectroscopy

We performed ^{13}C-CPMAS NMR spectroscopy with a Bruker DSX 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany) to study the chemical composition of selected incubated samples. A spinning speed of 6.8 kHz and a contact time of 1 ms were applied. A ramped ^{1}H pulse was used to prevent Hartmann-Hahn mismatches. The delay time ranged from 400 ms for clay MIN to 1 s for the LFs. Chemical shifts were assessed in reference to tetramethylsilane (=0 ppm). The obtained spectra were integrated according to the following chemical shift regions (Knicker et al., 2005): alkyl C ((−10)–45 ppm), O/N-alkyl C (45–110 ppm), aryl/olefin C (110–160 ppm), and carbonyl/carboxyl/amide C (160–220 ppm). Alkyl C to O/N-alkyl C ratios were calculated according to Baldock et al. (1997). They proposed this ratio as a sensitive index for the extent of decomposition of SOM.

2.5. Ammonium and nitrate

After each sampling, 5 g of dry mass equivalent of each fraction was extracted with 30 ml of 0.01 M $CaCl_2$ (1:6) by shaking for 2 h on a rotary shaker at 120 rpm (Certomat MOII, Sartorius, Göttingen, Germany) and then vacuum-filtrated using a cellulose nitrate filter (0.45 μm pore size, Sartorius, Göttingen, Germany). The low concentration of 0.01 M $CaCl_2$ was chosen to simulate a natural soil solution. Concentrations of NO_3^-–N and NH_4^+–N were measured in the extracts using a Skalar San^{++} Continuous Flow Analyzer (Breda, the Netherlands) to determine N_{min}.

2.6. Salt-extractable and microbial biomass organic carbon and nitrogen

To study the development of the microbial biomass carbon (MBC) and nitrogen (MBN) during our incubation experiment, we used the fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987; Joergensen et al., 1995; Joergensen, 1996). Fumigated moist subsamples (corresponding to 5 g dry weight of each fraction respectively bulk soil) were extracted (30 ml of 0.01 M $CaCl_2$; see Section 2.5) including dead cell material of former microorganisms. As an untreated subsample, we used the same extracts as for ammonium and nitrate determination.

After filtration, all extracts from fumigated and non-fumigated samples were frozen to −18 °C for later analysis of total organic carbon and total nitrogen bound with a DIMATOC 2000 (Dimatec Analysen GmbH, Essen, Germany). In displaying the data, we converted our carbon and nitrogen values to microbial biomass equivalents by dividing the measured concentrations by $k_{EC} = 0.45$ and $k_{EN} = 0.54$, respectively (Brookes et al., 1985; Vance et al., 1987). The control values of the non-fumigated samples were used as approximated values for salt-extractable organic carbon (SEOC) and nitrogen (SEON), respectively.

2.7. C/N analysis

Soil material from all samples was dried at 60 °C and further homogenized using a vibrating ball mill (Pulverisette 23, Fritsch, Idar-Oberstein, Germany). Total carbon and nitrogen were measured in duplicate by dry combustion (Euro EA 3000 Dual, EuroVector SpA, Milan, Italy). Carbonate measurements were performed according to Bimüller et al. (2014) using a calcimeter (08.53 Calcimeter, Eijkelkamp, Giesbeek, the Netherlands). Organic carbon was calculated by subtracting the inorganic carbon from total carbon.

2.8. CO_2–C and NH_3–N production

The same randomly selected subset of three replicates per fraction and bulk soil was used for monitoring CO_2–C and NH_3–N evolution each week. These triplicate incubation vessels were placed in separate air-tight 0.9 L glass jars with two beakers, one containing 20 ml of 0.1 M NaOH and the other one containing 10 ml of 2% boric acid. Samples were conditioned with these amendments in their jars for 7 d prior to the measurement of CO_2 and NH_3 evolution. The NaOH beakers were removed regularly once a week during incubation for determination of CO_2 adsorption, and replaced with fresh NaOH. CO_2 efflux from the substrates was measured for each chamber once a week. Evolved CO_2 precipitated in NaOH as Na_2CO_3, which was removed by 2 ml of 0.5 M $BaCl_2$ (Anderson and Ingram, 1993; Alef and Nannipieri, 1995; Luxhøi et al., 2006). The remaining, unconsumed NaOH was titrated to pH 8.3 using 0.1 M HCl. We used an automatic titrator (DL 28, Mettler-Toledo, Gießen, Germany). The three control jars contained no soil but 20 ml of 0.1 M NaOH and 10 ml of 2% boric acid. Microbial respiration was calculated as the difference in CO_2 adsorption between treatments (present as fractions or bulk soil, respectively) and control.

Evolved NH_3 was trapped in parallel using 2% boric acid (Kjeldahl, 1883) and titrated to pH 4.6.

2.9. Data analysis and statistics

CO_2–C respiration rates were modeled with a two-component exponential decay:

$$C(t) = C_r \exp(-k_r \cdot t) + C_s \exp(-k_s \cdot t) \quad (1)$$

where $C(t)$, the amount of respired carbon at time t, is split into mineralization from two pools differing in decomposability, with C_r being a modeled constant of the faster mineralizable carbon pool, C_s the modeled constant of the slowly mineralizable carbon pool, k_r and k_s the corresponding rate coefficients, and t the time of incubation (Stemmer et al., 1999; Collins et al., 2000). While the first term characterizes a faster, more active cycling soil organic carbon (SOC) pool, the second term indicates a slower, more passive cycling SOC pool (von Lützow et al., 2007; von Lützow et al., 2008). We set both terms to be equal to compare their output to total respiration and evaluate predominance.

Rates of CO_2–C, NH_3–N, and N_{min} production are expressed per gram SOC and total nitrogen (N_{tot}) initially present and do not account for soil carbon or even nitrogen losses over the incubation period (Weintraub and Schimel, 2003). A sum of fractions for selected parameters was calculated at the end of the data analysis by recombining the fractions according to the fraction mass distribution of the bulk soil (Table 1). MBC was determined and related to SOC contents of the different fractions after defined periods of incubation. All results are expressed per unit of incubated substrate, regardless of admixtures with quartz sand.

Statistical analysis was done using the statistical computing environment R 3.0.1 (R Core Team, 2013). We fitted a regression model to analyze the influence of substrate type and time on the different parameters (Appendix 3).

Our main interest was the effect of substrate type on the level of the parameter or the logarithmized parameter. The interaction with time was just considered to properly compensate for the time effect. We analyzed all pairwise comparisons between the bulk soil and all the fractions in a multiple comparisons procedure (Tukey test). Each test was carried out at an α-level of 0.05. In the figures, all error bars indicate the standard error of the mean.

Finally, a simple linear regression model was used to describe the linear dependence between carbon and nitrogen mineralization. We fitted a separate model for each substrate.

3. Results

3.1. Soil organic matter properties of bulk soil and various particle size fractions

The fractionation of 1 g bulk soil resulted in a mass distribution of 132.2 mg sand, 295.2 mg silt, and 572.6 mg clay (Table 1). A decrease in organic carbon content was detected from sand-sized (91.05 mg g^{-1}) to silt-sized (66.51 mg g^{-1}) and clay-sized fractions (55.13 mg g^{-1}), and the opposite trend was seen in the nitrogen concentration (2.94 mg g^{-1} in sand-sized and 5.36 mg g^{-1} in clay-sized fractions). High contents of POM, visible to the unaided eye, were responsible for great organic carbon contents in the sand-sized fraction. Inorganic carbon contents in the sand fraction were attributed to fragments of limestone. These carbonate concentrations were monitored over the experimental period and did not change substantially. A clear decrease in OC/N ratios from sand (30.90) to the finer particle size fractions (clay: 10.29) was detected (Table 1). The acidity of the incubated substrates mixed with quartz sand was neutral, ranging from pH values of 6.2 (silt) to 7.5 (clay). The sand substrate had a pH value of 6.9, the bulk soil mixture a pH of 7.2. The highest values of SEOC (Fig. 1a) and SEON (Fig. 1b) were detected in the clay fraction, and these exceeded MBC (Fig. 2a) and MBN (Fig. 2b), respectively. SEOC tended to decrease in the clay fraction over the course of the incubation, whereas a clear increase of SEOC was detected in the sand fraction (Fig. 1a).

The subsequent density fractionation of incubated particle size fractions revealed differences in the SOC distribution between SOM in LF and mineral-associated SOM (MIN). Ninety-five percent of the SOC in the sand-sized fraction was present as LF. In contrast, nearly all SOM in the clay fraction was associated with mineral particles, as the obtained LF material was below the detection limit. Forty-eight percent of the SOC in the silt fraction was stored in the LF. The chemical composition as demonstrated by the ^{13}C-NMR spectra (Fig. 3) showed a clear difference between major pools of SOC for all fractions. A large amount of fresh SOM rich in O/N-alkyl C dominated the LF component of the sand fraction. A higher degree of decomposition in the silt-sized LF was indicated by the higher alkyl C to O/N-alkyl C. The clay MIN fraction was also dominated by O/N-alkyl C.

3.2. Soil organic matter mineralization in bulk soil and various particle size fractions

Mineralization rates expressed per gram SOC and N_{tot} were calculated by using initial means of each fraction for all sampling dates. The 280-d cumulative respiration rates differed substantially among the fractions. The sand fraction showed the highest CO_2–C respiration rate per time unit (Fig. 4). The greatest release of CO_2–C (Fig. 5b) was observed from the sand fraction, which released 104.3 ± 3.2 mg CO_2–C (g SOC)$^{-1}$ after 280 d of incubation. The two finer fractions (silt and clay) mineralized significantly less CO_2–C (44.4 ± 2.7 and 87.9 ± 3.8 mg CO_2–C (g SOC)$^{-1}$). The bulk soil had respired 74.8 ± 2.5 mg CO_2–C (g SOC)$^{-1}$ after 280 d. By fitting a two-component exponential decay model to the respiration curves of each fraction, it was possible to differentiate between a rapidly mineralizable carbon (C_r) pool and a more slowly mineralizable carbon (C_s) pool. The first 7–12 d were dominated by consumption of C_r in the sand and bulk soil, whereas the basal respiration of C_s dominated during the later periods of the incubation experiment in these substrates (Fig. 4). The rapid pool of the clay fraction lasted longer: until day 85. In the silt fraction, mineralization rates were exclusively dominated by the slow pool. When the easily accessible fractions of SOC were respired, the carbon decomposition rate decreased exponentially. The flat tail of the graph is governed by mineralization of more stable organic material, whereas the fast primary decay is controlled by decomposition of the smaller, declining rapid pool.

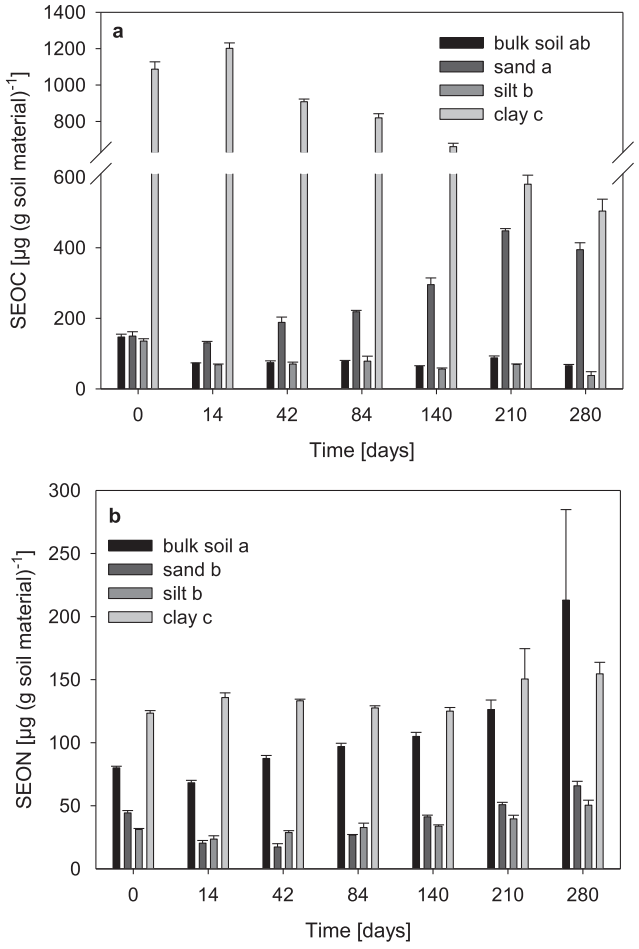


Fig. 1. (a) Salt-extractable organic carbon (SEOC) and (b) salt-extractable organic nitrogen (SEON) per gram soil material. Values are means of three replicates with standard errors. A transformation of the response variable was needed in the case of SEOC to satisfy the assumptions of a linear regression model. Different lowercase letters indicate significant differences between measured substrates (α-level = 0.05).

The ANOVA revealed significant differences (α-level = 0.05) in CO_2–C respiration between all fractions (calculated per gram SOC) compared to each other and the bulk soil after 280 d of incubation (Fig. 5b). Calculated per gram fraction, bulk soil and clay did not differ significantly (Fig. 5a). On a per SOC basis, the clay fraction mineralized over 80% of the CO_2 mineralized by the sand fraction (Fig. 5b) whereas on a mass soil basis, the clay fractions mineralized about half of the CO_2 mineralized by the sand fraction. Mineralization rates of the rapid pools (k_r) of the different sample sets increased from silt (k_r = 0.0380 d^{-1}) to clay (k_r = 0.0392 d^{-1}) to bulk soil (k_r = 0.0410 d^{-1}) and sand (k_r = 0.0449 d^{-1}). Therefore, C_r of the sand fraction indicated the fastest decrease in carbon mineralization rates. The mineralization of C_r of the clay fraction dominated respiration during the first phase of the incubation until day 85, because of its relatively large pool size and low mineralization rates (Fig. 4). We mathematically recombined the total respired CO_2–C per gram fraction and per gram SOC from the

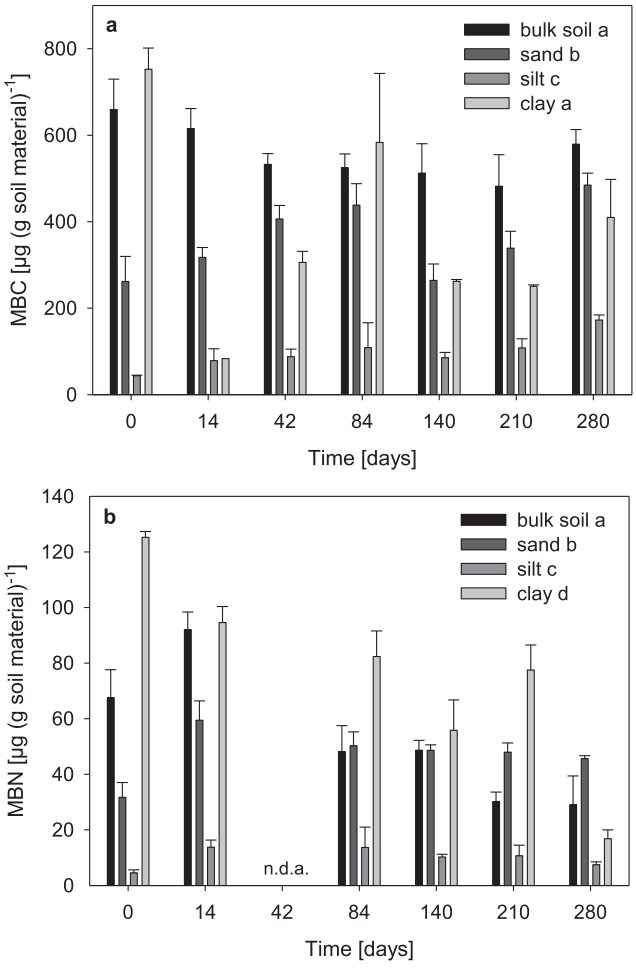


Fig. 2. (a) Microbial carbon (MBC) and (b) microbial nitrogen (MBN) per gram soil material. Values are means of three replicates with standard errors. Different lowercase letters indicate significant differences between measured substrates (α-level = 0.05). n.d.a.: no data available.

cumulative respiration values of each fraction according to the mass proportions of each fraction from the bulk soil (Table 1). The respired CO_2–C rates of the bulk soil and the recombined fractions were neither significantly different per gram substrate nor per SOC (α-level = 0.05). Approximately 4.4% (silt) to 10.4% (sand) of initially available SOC was respired during the incubation, depending on soil fraction (Fig. 5b). The clay fraction respired 8.8% of initially available SOC until the end of the experiment, similar to the bulk soil (7.5%).

In all substrates nitrogen was mineralized at a low level at the beginning of the experiment; nitrogen mineralization increased over the experimental period and highest N_{min} concentrations were measured at the end of the experiment (Table 2). Net nitrogen mineralization during incubation was higher in clay than in silt and sand. Generally, more nitrogen was mineralized as NH_4^+-N than as NO_3^-–N. Cumulative NH_3–N losses increased continuously with a linear trend for all incubated substrates. The greatest NH_3–N production was measured in the clay fraction and the bulk soil (Fig. 6), which were not significantly different from each other at the end of the experiment. These two substrates (mixed with quartz sand) had the highest pH values (>7), leading to a greater probability of NH_3–N release. Likewise, we did not observe a significant difference between silt and sand after 280 d, which both (mixed with quartz sand) had a pH value <7. The mathematical recombination of NH_3–N production in the fractions differed significantly from that in the bulk soil.

The relationship between cumulative carbon and nitrogen mineralization showed a linear trend for all four sample materials (Fig. 7). The gradient was highest for the bulk soil, which means that all individual fractions as well as the mathematical recombined sum of all fractions mineralized less nitrogen per unit CO_2–C compared to the bulk soil. The sand had the lowest N_{min} concentration per mineralized carbon.

3.3. Microbial biomass

Whereas MBC hardly changed during the experimental period, MBN decreased over time in the bulk soil and clay fraction (Fig. 2). MBC and MBN were lowest in the silt fraction. The clay fraction showed the strongest MBC dynamics and the highest MBN values, except for the last sampling day. MBC in the clay fraction recovered somewhat after an initial drop (Table 4). An overall maximum of 125.2 µg N g^{-1} clay and 752.6 µg C g^{-1} clay in the microbial biomass was observed at 0 d (Fig. 1b). The metabolic quotient showed that substrate availability had decreased by the end of the experiment (Table 3). Microbial carbon mineralization became more efficient during the incubation, as the metabolic quotients generally decreased in all fractions. Mathematical recombination of the fractions calculated from the metabolic quotients revealed values more than twice that of the bulk soil. Carbon bioavailability of organic matter was generally greater in the fractions as compared to that of bulk soil, demonstrating that carbon mineralization in soil fractions was less efficient than in the bulk soil. The strongest dynamics with regard to the metabolic quotient were in the clay

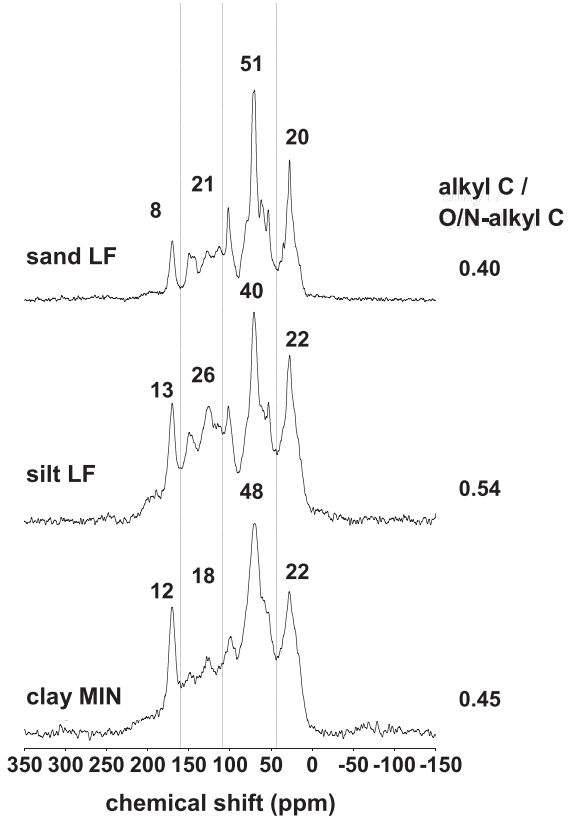
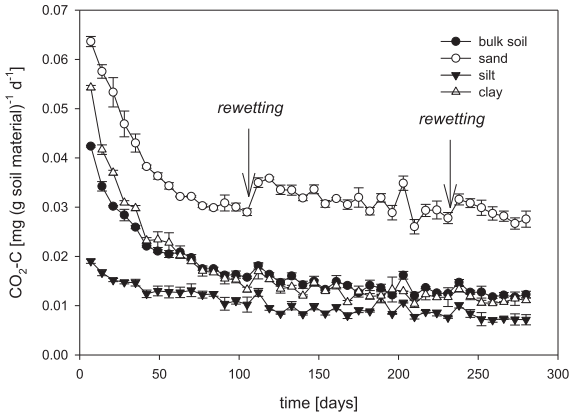


Fig. 3. Chemical composition of selected density fractions after subsequent density fractionation of the incubated substrates. The composition is given as relative signal intensity of the integrated chemical shift regions (alkyl C, (−10)–45 ppm; O/N-alkyl C, 45–110 ppm; aromatic C, 110–160 ppm; carbonyl/carboxyl/amide C, 160–220 ppm) derived by ^{13}C-CPMAS NMR spectroscopy. Numbers above the spectra indicate the relative intensity (%) for the single shift regions. LF: light fraction; MIN: mineral-bound SOM.



Double exponential decay with four parameters

$$C(t) = C_r e^{(-k_r \times t)} + C_s e^{(-k_s \times t)}$$

Parameters	Bulk soil	Sand	Silt	Clay
C_r	0.0301	0.0454	0.0072	0.0469
k_r	0.0410	0.0449	0.0380	0.0392
C_s	0.0190	0.0328	0.0132	0.0172
k_s	0.0017	0.0004	0.0020	0.0017
R^2	0.9588	0.8905	0.7681	0.9657
Half-life of C_r [days]	16.91	15.44	18.24	17.68
Half-life of C_s [days]	407.73	1732.87	346.57	407.73
C_r [% of carbon respired]	61.30	58.06	35.29	73.17
C_s [% of carbon respired]	38.70	41.94	64.71	26.83
$C_{respired}$ after 280 d [mg g^{-1}]	4.85	9.50	2.95	4.85
Initial C_{total} [mg g^{-1}]	60.54	85.92	61.90	52.99
C_r [% of $C(t)$]	4.91	6.42	1.68	6.69
C_s [% of $C(t)$]	3.10	4.64	3.08	2.46
Slow pool dominates respiration after day	11.71	6.89	0.00	85.32

Fig. 4. CO_2–C respiration time courses from a 280-d laboratory incubation of different soil particle size fractions of a Rendzic Leptosol. Values are means of three replicates with standard errors (expressed per gram soil material and day). $C(t)$: the amount of respired carbon at time t, C_r: modeled constant of the rapidly mineralizable carbon pool, C_s: modeled constant of the more slowly mineralizable carbon pool, k_r and k_s: corresponding rate coefficients, t: time of incubation.

fraction, which showed the greatest decrease. Regarding the ratio of MBC to SOC, the recombined sum of fractions had less MBC compared with the bulk soil (Table 4), which showed hardly any variation over the course of the experimental period.

4. Discussion

4.1. Carbon and nitrogen mineralization differed among particle size fractions

Carbon mineralization based on mass of fractions and carbon contents (Fig. 5) followed the order sand > clay > silt, which is in line with the findings by Parfitt and Salt (2001) and Christensen (1987). Highest respiration rates for the sand fraction, containing high amounts of POM, were consistent with other studies, which ascribe fragments of plant material as readily available substrates that are rapidly mineralized (Hassink, 1995; Christensen, 2001). We considered CO_2 production rates to solely represent respired carbon from organic carbon mineralization. Carbonate weathering was neglected in all substrates due to almost absent carbonates (Table 1).

The similar courses of the CO_2–C release curves from the bulk soil and that of recombined fractions (Fig. 5) are in line with the results of Swanston et al. (2002), who did not find significant differences between the cumulative carbon mineralization of bulk soil and the summed fractions. While Mueller et al. (2012) found a higher carbon mineralization after fractionation, Crow et al. (2007) found a lower cumulative respiration from summed fractions compared to the bulk soil. Gregorich et al. (1989) explained such controversial findings by referring to methodological aspects. They reported a relation between the energy input during fractionation and the carbon respiration behavior of the summed fractions compared to the bulk soil.

In contrast to carbon mineralization, nitrogen mineralization increased with decreasing particle size (Table 2; Christensen and Olesen, 1998), because finer particles have a narrower C/N ratio, a greater proportion of mineralizable nitrogen, and a higher surface-to-volume ratio than coarser particles (Chichester, 1969; Cameron and Posner, 1979; Parfitt and Salt, 2001). From this data it becomes obvious that the mineralizable nitrogen in the clay fraction is in excess of that required by microorganisms present in this fraction. Our experiment clearly indicates that enough nitrogen was available in the clay fraction, as demonstrated by the high N_{min} contents (Table 2) and NH_3–N losses (Fig. 6) until the end of the incubation. At the same time, this nitrogen obviously could not be immobilized by microorganisms. The decomposer system in the clay fraction seemed to be strongly carbon-limited (Parfitt and Salt, 2001), as indicated by rapidly declining carbon mineralization rates (Fig. 4), SEOC contents (Fig. 1a), and MBN contents (Fig. 2b).

4.2. SOM availability in various particle size fractions

^{13}C-NMR data of sand-sized LF (Fig. 3) showed relatively high O/N-alkyl C contents and a low alkyl C to O/N-alkyl C ratio. These values correspond to slightly decomposed beech litter (Kögel-Knabner, 2002), demonstrating that the organic matter in the sand-sized fraction was of rather fresh nature. This was consistent with the high CO_2–C respiration rates of the sand (Fig. 5), representing the most bioavailable SOM fraction. In comparison, SOM in the mineral-bound clay fraction (clay MIN; Fig. 3) was also dominated by O/N-alkyl C indicating an enrichment of polysaccharides over the aryl C structures. A low alkyl C to O/N-alkyl C ratio of the mineral-bound SOM in the clay fraction has been considered to indicate a low recalcitrance (Kleber et al., 2011). Our data show that the organic matter in the clay fraction, although dominated by O/N-alkyl C components such as polysaccharides is obviously much less bioavailable and/or accessible for the microbial biomass. This becomes evident from rapidly declining carbon mineralization rates (Figs. 4 and 5), SEOC contents (Fig. 1a), and MBN contents (Fig. 2b). Consequently, the relative intensities of the O/N-alkyl C and the alkyl C to O/N-alkyl C ratio were insufficient to draw conclusions regarding bioavailability of SOM in mineral-associated fractions.

Generally, the amounts of MBC and MBN are rather low compared to other German forest soils dominated by beech (Joergensen et al., 1995). We also assume a relocalization of soluble organic carbon and nitrogen, as well as microbial remnants from coarser to finer fractions. Our observed results for the bulk soil concerning the SOC stored in MBC (Table 4) and the metabolic quotient (Table 3) are in line with the literature on beech forest soils (Anderson and Domsch, 1993). The metabolic quotient was higher

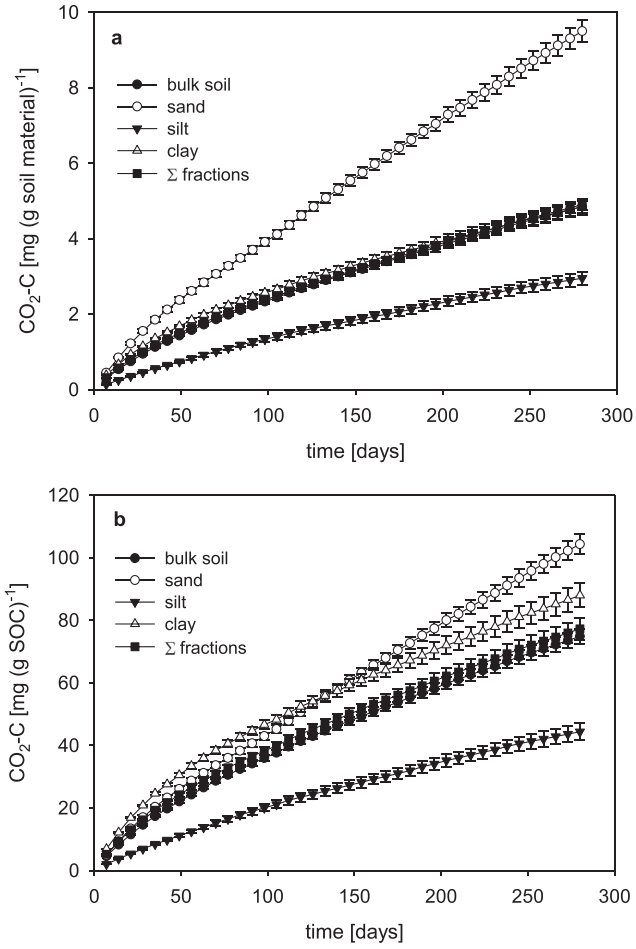


Fig. 5. Cumulative CO_2–C respiration (a) per gram soil material and (b) per gram mean initial soil organic carbon (SOC) of each fraction and the bulk soil, respectively. Values are means of three replicates with standard errors.

at the beginning of the incubation among all fractions and the bulk soil (Table 3) due to greater masses of labile material still present in the samples (Creamer et al., 2013). These substrates were consumed during the early incubation phase, resulting in a decrease of the metabolic quotient with time. The lower metabolic quotients (Table 3) of the bulk soil in comparison to each single fraction implied a better bioavailability of SOM after fractionation in comparison to occluded SOM protected within the bulk soil. Hence, intact bulk soil structure allowed for a more efficient microbial carbon mineralization. Except for the first flush, carbon mineralization per unit total microbial biomass (Table 3) was lower in the clay fraction than in the silt and sand fraction, because of a greater protection of the organic matter in the finer textures (Hassink, 1995) with narrower C/N ratio (Erickson et al., 2001). We also attribute the very high metabolic quotients in the clay fraction at the beginning to a consequence of the fractionation procedure. Clay isolation from the aqueous suspension was the last step during the fractionation procedure, which delivered dissolved organic matter (Fig. 1) to this fraction (Gregorich et al., 1989; Parfitt and Salt, 2001; Mueller et al., 2012). The higher metabolic quotients in the fractions imply a higher energy release by the microbial biomass compared to that released in the bulk soil. We therefore conclude that the availability of carbon controlled the mineralization pattern in the fractions: nitrogen immobilization was inhibited in the clay fraction because carbon was limited (Parfitt and Salt, 2001). This was indicated by a strong decline in carbon mineralization and the metabolic quotient, an accumulation of N_{min}, and high NH_3–N losses. A reverse pattern was found in the sand fraction, where mineralizable carbon was available but a nitrogen limitation occurred. Similar conditions have been observed for decaying litter (Recous et al., 1995; Henriksen and Breland, 1999; Erickson et al., 2001; Jensen et al., 2005). This positive correlation between C mineralization and C/N ratios has already been identified for the bulk soil (Erickson et al., 2001), and is complemented by our findings concerning the soil fractions, with the clay fraction governing the bulk soil. Nearly unchanged alkyl C to O/N-alkyl C ratios from sand to clay were accompanied by a narrowing C/N ratio pointing to the fact that decomposition has occurred. Our results clearly show that the C/N ratio as well as the alkyl C to O/N-alkyl C ratio of the clay fraction cannot be used as an indicator of biological decomposability in mineral soil fractions. These parameters may be useful indicators for decomposition of plant residues on or also in the mineral soil, but they should not be used for mineral-associated SOM.

4.3. Decoupled carbon and nitrogen mineralization

Within each fraction and the bulk soil, we observed good positive correlations between the amounts of nitrogen mineralized with the total amounts of carbon mineralized. A lag in nitrogen mineralization for the sand fraction was observed (Fig. 7), as indicated by the gentle slope of the linear regression. This lag can be explained by the larger C/N ratio in comparison with the other fractions and a labile carbon-rich organic matter pool in this fraction, which must first be exhausted before microbes become carbon-starved and start mineralizing nitrogen (Waksman, 1927; Aber and Melillo, 2001; Weintraub and Schimel, 2003). This went along with a decrease in CO_2–C respiration before nitrogen mineralization started. In comparison, bulk soil showed relatively high ratios between mineralized carbon and nitrogen (Fig. 7). The ratio of carbon to nitrogen mineralization (Fig. 7) followed the order sand > silt > clay. This sequence is in line with the results by Parfitt and Salt (2001). When comparing Table 2 and Fig. 6, nitrogen mineralization in the clay fraction corresponded to the bulk soil. This confirms the dominant role of the clay fraction within the studied soil and its prominent part as an obvious nitrogen source for mineralization. Silt and sand fractions hardly played a role in nitrogen mineralization, while sand was dominating the carbon mineralization. The recombined particle size fractions, which were ultrasonicated and freeze-dried, mineralized less nitrogen per unit respired carbon compared to the undisrupted bulk soil. This deficit in nitrogen mineralization of recombined fractions was also observed by Swanston et al. (2004). Fresh mineral surfaces are

Table 2
Nitrogen mineralization time courses during 280-d laboratory incubation of soil particle size fractions and the bulk soil. N_{min} (sum of ammonium and nitrate) values are means of three replicates expressed per initial N_{tot} (total nitrogen) with standard errors. Different lowercase letters indicate significant differences between measured substrates (α-level = 0.05). Different capital letters indicate a significant difference between bulk soil and the mathematical recombination of all three fractions (α-level = 0.05).

Day	N_{min} [mg (g N_{tot})$^{-1}$]				
	Bulk soil A a	Sand b	Silt b	Clay c	∑ Fractions B
0	13.9 ± 0.3	10.8 ± 0.8	6.2 ± 0.2	9.7 ± 0.2	8.8 ± 0.2
14	15.1 ± 0.5	4.4 ± 0.7	6.3 ± 0.2	11.8 ± 0.3	9.2 ± 0.2
42	16.5 ± 0.3	2.0 ± 0.3	6.5 ± 0.4	12.8 ± 0.5	9.5 ± 0.3
84	17.8 ± 0.2	3.4 ± 0.2	6.8 ± 0.3	14.5 ± 0.2	10.8 ± 0.2
140	21.3 ± 0.8	7.5 ± 0.4	7.9 ± 0.3	16.5 ± 0.4	12.8 ± 0.3
210	24.1 ± 1.5	8.1 ± 0.3	9.9 ± 0.9	18.9 ± 0.6	11.2 ± 4.6
280	29.3 ± 3.1	11.3 ± 0.5	13.9 ± 2.5	25.4 ± 3.4	20.1 ± 1.5

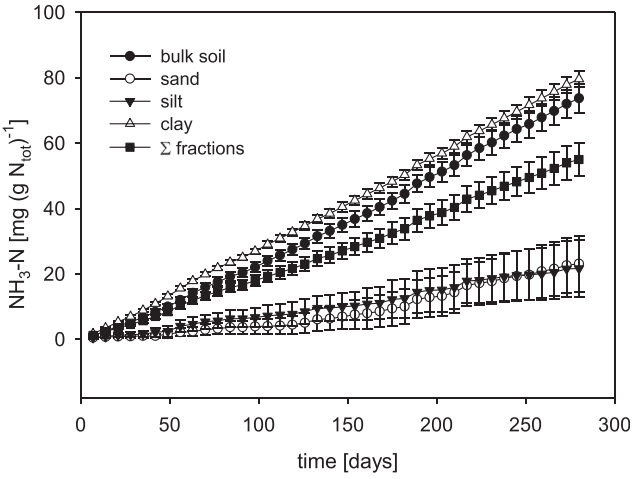
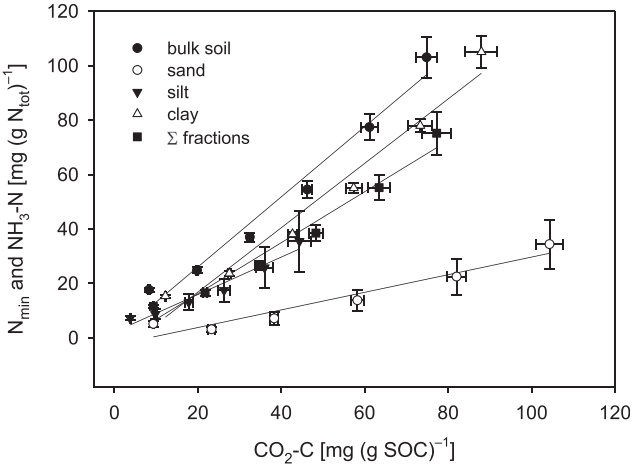


Fig. 6. Cumulative NH_3–N production per gram mean initial soil organic nitrogen (N_{tot}) of each fraction and the bulk soil. Values are means of three replicates with standard errors. N_{tot}: total nitrogen.

considered to specifically bind nitrogen-rich organic matter (Sollins et al., 2006; Kleber et al., 2007; Dümig et al., 2012; Pronk et al., 2013). We assume that increasing the surface area by fractionation provided accessory mineral surfaces allowing for additional binding of nitrogen-rich compounds, in addition to ammonium fixation via cation exchange.

The quantity of available inorganic nitrogen is critical for mineralization (Recous et al., 1995; Stemmer et al., 1999). The sand fraction was hindered from nitrogen immobilization and carbon mineralization by a shortage of available mineral nitrogen. The steep trend of the NH_3–N release by the clay fraction showed a high availability of net mineralized nitrogen (Fig. 6), parallel to the CO_2–C emission of this fraction. In comparison, sand emitted high amounts of CO_2–C and only small amounts of NH_3–N, similar to the silt fraction. Generally, substrates with higher pH had a greater potential for NH_3–N volatilization. In terms of nitrogen dynamics,



	Bulk soil	Sand	Silt	Clay	∑ Fractions
a	0.29	-2.65	2.17	-6.82	-2.49
b	1.29	0.32	0.69	1.18	0.94

Fig. 7. Sum of nitrogen mineralized (expressed per gram initial N_{tot}) and NH_3–N production regressed against cumulative carbon mineralized (expressed per gram initial SOC). Values are means with standard errors. The table presents the coefficients of the linear regressions. N_{min}: sum of ammonium and nitrate; N_{tot}: total nitrogen.

Table 3
Metabolic quotient. Calculated values are a combination of three replicates of CO_2–C and three replicates of microbial carbon (MBC). Different lowercase letters indicate significant differences between measured substrates (α-level = 0.05). Different capital letters show a significant difference between bulk soil and the mathematical recombination of all three fractions (α-level = 0.05).

Day	CO_2–C [mg (g MBC)$^{-1}$ h^{-1}]				
	Bulk soil A a	Sand b	Silt b	Clay c	∑ Fractions B
14	2.3 ± 0.1	7.6 ± 0.3	13.2 ± 3.2	20.8 ± 0.5	16.8 ± 1.3
42	1.7 ± 0.0	4.0 ± 0.2	6.6 ± 0.9	3.2 ± 0.1	4.3 ± 0.4
84	1.4 ± 0.0	2.9 ± 0.2	10.7 ± 3.3	1.4 ± 0.2	4.3 ± 1.1
140	1.2 ± 0.1	5.3 ± 0.4	4.2 ± 0.3	1.9 ± 0.0	3.0 ± 0.2
210	1.1 ± 0.1	3.3 ± 0.2	3.2 ± 0.3	1.7 ± 0.1	2.4 ± 0.1
280	0.9 ± 0.0	2.4 ± 0.1	1.7 ± 0.1	1.2 ± 0.1	1.5 ± 0.1

fractionation reduced the mineralization. However, once isolated and no longer interacting with one another within the intact soil matrix, the fractions do not mineralize as they do in the bulk soil (Sollins et al., 1984; Swanston et al., 2002). The incubation of the particle size fractions revealed that the mineralization of nitrogen in soils is decoupled from carbon mineralization.

5. Conclusions drawn from fractionation and subsequent incubation

Studying decomposition and biodegradability of specific SOM compartments, such as the respiration and mineralization behavior of the microbial biomass in the different particle size fractions, allowed us to evaluate the stability of SOM associated with these individual fractions. Fractionation lowered the efficiency of carbon mineralization, demonstrated by higher metabolic quotients in the fractions. Thus, intact bulk soil structure allowed for a more efficient microbial carbon mineralization.

The actual bioavailability of carbon and nitrogen controls the mineralization pattern in the different SOM fractions. The clay fraction is characterized by inhibited nitrogen immobilization due to carbon limitation, as the high proportions of potentially labile carbohydrates are not bioavailable in the mineral-associated form. In the sand fraction, which contains a litter-like POM fraction, carbon is available for mineralization, but nitrogen is limited. These results also imply that the relative intensities of the O/N-alkyl C and the alkyl C to O/N-alkyl C ratio are not suitable to draw conclusions regarding decomposition of SOM in mineral-associated fractions. Whereas these parameters are useful for decaying plant residues, they do not indicate the bioavailability and accessibility of these

Table 4
Microbial carbon (MBC) related to the soil organic carbon (SOC) content at each sampling date. A transformation of the response variable was needed to satisfy the assumptions of a linear regression model. Different lowercase letters indicate significant differences between measured substrates. Different capital letters show a significant difference between bulk soil and the mathematical recombination of all three fractions (α-level = 0.05).

Day	MBC [mg (g SOC)$^{-1}$]				
	Bulk soil A a	Sand b	Silt c	Clay a	∑ Fractions B
0	10.1 ± 0.9	2.9 ± 0.5	0.7 ± 0.0	13.6 ± 0.9	8.4 ± 0.6
14	8.4 ± 0.8	3.6 ± 0.2	1.1 ± 0.4	1.4 ± N/A	1.6 ± 0.1
42	9.0 ± 0.5	4.5 ± 0.5	1.4 ± 0.3	5.5 ± 0.6	4.2 ± 0.5
84	8.5 ± 0.5	5.3 ± 0.6	1.7 ± 0.9	10.6 ± 2.8	7.3 ± 2.0
140	8.5 ± 0.9	3.1 ± 0.4	1.4 ± 0.2	5.0 ± 0.1	3.7 ± 0.2
210	10.2 ± 2.0	4.6 ± 0.4	2.2 ± 0.4	5.6 ± 0.4	4.5 ± 0.4
280	10.1 ± 0.4	5.6 ± 0.3	2.9 ± 0.2	8.1 ± 1.6	6.2 ± 1.0

N/A: data not available.

components for microorganisms in mineral-associated fractions. There is clear evidence that carbon and nitrogen mineralization are closely coupled processes during the decay of plant residues on or in the soil. Our results suggest that they are decoupled in the mineral-associated fractions of the soil, as the interactions of both carbon and nitrogen containing components with the mineral matrix strongly modulate the mineralization dynamics. We therefore conclude that the C/N ratio as well as the alkyl C to O/N-alkyl C ratio cannot be used as a well-founded indicator for decomposability of plant residues when characterizing soil fractions that are stabilized by spatial inaccessibility and by organo-mineral interactions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.08.001.

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Publications

Peer-reviewed publications

Bimüller C, Mueller C W, von Lützow M, Kreyling O, Kölbl A, Haug S, Schloter M, Kögel-Knabner I (2014): Decoupled carbon and nitrogen mineralization in soil particle size fractions of a forest topsoil. **Soil Biology and Biochemistry** 78, 263-273.

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Presentations

Oral presentations

Tejedor J, Gasche R, Gschwendtner S, Leberecht M, **Bimüller C**, Kögel-Knabner I, Polle A, Schloter M, Rennenberg H, Simon J, Hanewinkel M, Baltensweiler A, Bilela S, Dannenmann M (2014): Climate change impairs processes of soil and plant N cycling in European beech forests on marginal soil. General Assembly 2014 of the European Geosciences Union in Vienna, Austria, 27.04.-02.05.2014.

Bimüller C (2014): Vom Blatt zum Boden – Stickstoffverteilung und –stabilisierung in organischer Bodensubstanz. Institutskolloquium Physische Geographie Universität Erlangen, Germany, 23.04.2014, invited.

Bimüller C, Dannenmann M, Tejedor J, Gasche R, Buegger F, Schroll R, Bilela S, Rennenberg H, Kögel-Knabner I (2013): Welchen Einfluss hat Sommertrockenheit auf die Stickstoffverteilung einer Rendzina? Annual meeting of the Deutsche Bodenkundliche Gesellschaft 2013 in Rostock, Germany, 07.-12.09.2013.

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Poster presentations

Dannenmann M, TejedorJ, Bilela S, Gasche R, Gschwendtner S, Leberecht M, **Bimüller C**, Kögel-Knabner I, Polle A, Schloter M, Rennenberg H (2012): Gross nitrogen fluxes in intact beech-soil-microbe systems under simulated climate change. Eurosoil 2012 in Bari, Italy, 02.-06.07.2012.

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