Impacts of nitrogen deposition on litter and soil carbon dynamics in forests

Atmospheric Nitrogen Deposition to Global Forests

Kuyper, Thomas W.; Janssens, Ivan A.; Vicca, Sara

https://doi.org/10.1016/B978-0-323-91140-5.00012-9

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed using the principles as determined in the Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. According to these principles research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact
openaccess.library@wur.nl

Chapter 8

Impacts of nitrogen deposition on litter and soil carbon dynamics in forests

Thomas W. Kuyper[a], **Ivan A. Janssens**[b] and **Sara Vicca**[b]

[a]*Soil Biology Group, Wageningen University and Research, Wageningen, the Netherlands;* [b]*Plants and Ecosystems (PLECO), Biology Department, University of Antwerp, Wilrijk, Belgium*

1. Introduction

Due to enhanced nitrogen (N) deposition, many forests in the boreal and temperate regions, whose growth is usually N limited (LeBauer and Treseder, 2008), have increased their productivity. Tropical and subtropical forests can also be N limited, but are more often limited by phosphorus (P) or base cations (Cleveland et al., 2011; Kaspari et al., 2008; Vitousek, 1984). As a consequence of higher N availability, litter production has increased in many forests. Even under the assumption of no effect of N on soil carbon (C) dynamics, higher inputs in combination with unchanged decomposition rates result in a higher stock of soil organic C (SOC). During the last decades it has been suggested that an intended (through forest fertilization to increase productivity) or unintended (through atmospheric N deposition) consequence of higher N availability increases C sequestration in forests (Du and de Vries, 2023). Higher N availability furthermore increases the responsiveness to the growing atmospheric CO_2 concentration, providing a negative feedback to further increases in atmospheric CO_2. This beneficial effect of N addition on C sequestration is larger if N deposition also retards decomposition and soil C dynamics.

Recent reviews on C storage in (forest) soils were published by Basile-Doelsch et al. (2020), Cotrufo et al. (2021), and Hoffland et al. (2020). Major factors that influence C storage are (1) the recalcitrance (decomposability) of the inputs; (2) the extent of microbial modification of plant litter and the properties of microbial necromass; (3) the extent of mineral protection against degradation through associations of SOC with the mineral phase; and (4) priming effects, being the acceleration (or sometimes retardation) of SOC decomposition through exudates. A meta-analysis of priming effects (Feng and Zhu, 2021) suggests that addition of mineral N results in negative priming effects. However, as most priming studies typically last for a few months only, it is difficult to assess how such effects translate into longer-term SOC dynamics.

For a long time the effect of N on decomposition was considered positive as microbial decomposition was assumed to be nutrient-limited, and especially N-limited. This positive N effect almost reached the status of textbook knowledge. After Fog (1988) published his review, research focus shifted toward the rate-retarding effect of N on decomposition and SOC levels. The number of studies on the effects of N addition on soil C fluxes has been steadily increasing over the last decades. Our understanding of the mechanisms underlying these N effects has considerably increased. Nitrogen deposition has both direct effects on soil processes, and indirect effects, due to changes in litter quality, as manifested for instance in a lower litter C:N ratio or higher litter N:P ratio (Sardans et al., 2016), or due to changes in below-ground C allocation (Janssens et al., 2010). Nitrogen deposition impacts on saprotrophic soil organisms, through changes in species or functional-group composition, abundance and (functional) diversity, but also through changes in mycorrhizal fungal communities (Lilleskov et al., 2023). The saprotrophic community composition and activity are determined by both environmental characteristics (soil properties, climate) and the nature and chemical composition of the inputs, often captured under the concept of litter quality. Changes in the quality of litter inputs, either because chemical properties of litter change under the influence of N deposition or because N deposition leads to vegetation shifts to plant species with different litter qualities, are therefore connected to changes in these biotic communities. Changes in litter quality, combined with changes in saprotrophic communities, not only result in changes in the quantity but also in the quality

Atmospheric Nitrogen Deposition to Global Forests. https://doi.org/10.1016/B978-0-323-91140-5.00012-9

of SOC, as different C compounds are differentially affected by N addition (see Section 5.3). Litter and SOC quality changes are relevant for both C pools considered intrinsically recalcitrant and C pools that strongly interact with the mineral matrix. This latter factor therefore indicates a major role for soil properties, such as mineralogy, texture and pH.

Changes in microbial communities translate into changes in the gene pool of the soil biotic community and these can be assessed through genomics techniques. An intermediate step, through assessing changes in the abundance or activity of enzymes involved in the turnover of SOC and the acquisition of nutrients from the organic C pool, provides a more direct way to evaluate the impact of N deposition on soil processes. Measurements of soil respiration provide assessments of the changes in C cycling, but separation between autotrophic and heterotrophic respiration is complicated. Activity changes can also be detected by mass loss of litter confined in litter bags. These three factors (assessing diversity, activity or the genetic make-up of the soil biotic community) are of course connected, however the nexus between these three factors is not strong enough that measurement of one set can always be considered a reliable proxy for the other factors (Chen and Sinsabaugh, 2021). When considering the impacts of unintended N enrichment, it is relevant to distinguish between deposition of ammonium (NH_4^+) and nitrate (NO_3^-) with varying ratios over the globe (Schwede et al., 2023), as it is likely that these forms of N have differential impacts on microbial physiology, for instance the regulation of oxidative enzymes (Osono and Takeda, 2001).

In this review we provide a comprehensive overview of the effects of N deposition on litter and SOC dynamics. We focus on the saprotrophic community (species composition, abundance, and functional diversity), its links with processes like respiration and mass loss of organic material in relation to enzyme dynamics, and its links with the genetic make-up of that saprotrophic community. We focus on microbes (saprotrophic fungi and bacteria), but also briefly review changes in soil fauna. Changes in the mycorrhizal fungal community are dealt with, as their enzymatic capabilities make a link with soil organic matter dynamics (Lilleskov et al., 2023). We put the microbial community at the center of our review to achieve a mechanistic focus. We connect qualitative and quantitative changes in litter inputs to effects on those microbial communities and link those to differential sensitivity of various C compounds to N addition. Differential sensitivity of various C compounds is then linked to soil properties, to understand the extent to which different ecosystems are differentially affected by N deposition. A graphical summary of the topics that are dealt with, is provided in Fig. 8.1.

As noted above, reduction of organic matter decomposition and increase of soil C sequestration have been proposed as a beneficial side effect of N enrichment as enhanced C sequestration could mitigate the effects of climate change. At the same time, climate change (especially higher temperatures) impacts microbial physiology,

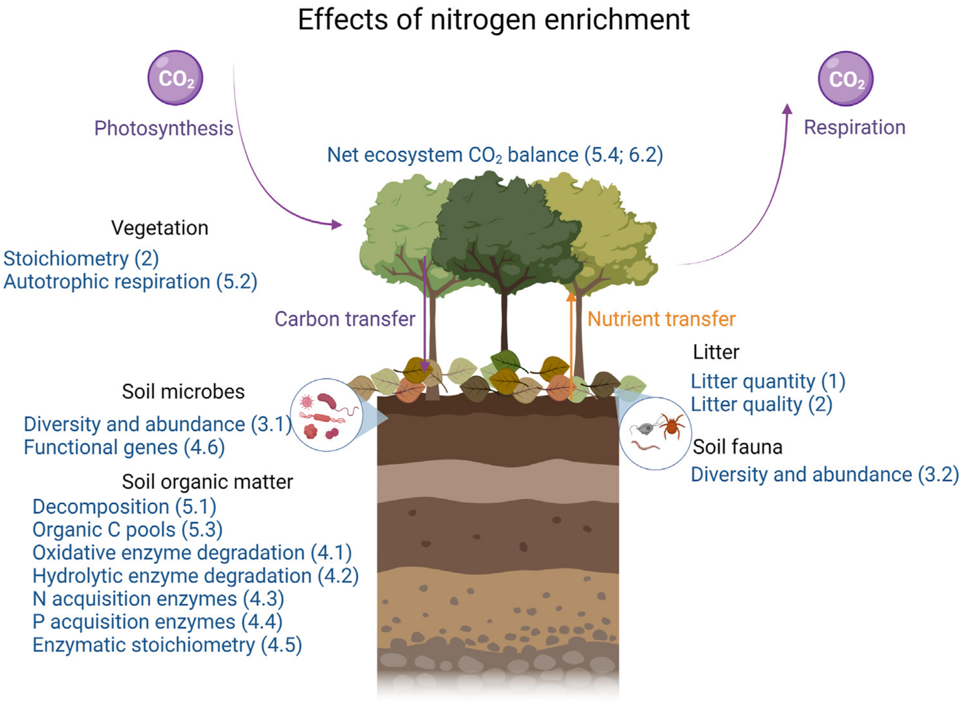

FIG. 8.1 Conceptual figure of the effects of N enrichment. The numbers within brackets indicate the sections where the topic is dealt with.

accelerating decomposition, so interactions between N enrichment and climate change are of potential concern. On the basis of the available data we speculate on the consequences of such interactions. Finally, in order to predict the long-term consequences of N addition on the global C cycle we cannot rely on empirical studies only. We discuss in which (mechanistic) ways N enrichment is incorporated in global models of C cycling. We end this review with a brief summary and an outlook, where we indicate methodological issues and major questions that have not been sufficiently addressed.

2. Impacts of nitrogen deposition on litter quality

As a consequence of N deposition, a gradual replacement of ectomycorrhizal trees by arbuscular mycorrhizal trees has been reported (Averill et al., 2018; Jo et al., 2019) a process to which both N deposition and global warming, which has a larger impact on N than on P availability, contribute. Changes in tree species composition alter the properties of leaf and needle litter, as aboveground litter from arbuscular mycorrhizal trees is decomposed at a higher rate than that of ectomycorrhizal trees, at least in temperate forests (Keller and Phillips, 2019). N-deposition driven shifts toward increasing dominance by arbuscular mycorrhizal trees, however, at the same time result in increased C accumulation in the mineral-associated organic matter pool, countering the aboveground effect (Keller et al., 2021). Tree species shifts therefore feedback to C cycling processes, but effects are poorly known.

Next to changes in tree species composition, N enrichment alters litter properties without plant species turnover. N enrichment generally increases foliage N mass fractions, and hence reduce C:N ratios, and this change modifies organic matter decomposition and N mineralization. Changes have been reported for both leaf and needle litter and for fine roots. A meta-analysis by Van Diepen et al. (2015) indicated that N addition significantly increased litter N mass fractions (+27%), but significantly reduced mass fractions of P (−7%), Ca (−20%), Mg (−11%), and Mn (−24%). The latter decline affects Mn dynamics and the enzymatic activity of Mn-dependent peroxidases, involved in the breakdown of lignin and lignin-like substances (Stendahl et al., 2017; Whalen et al., 2018). Effects were larger for higher N addition rates (>100 kg N ha^{-1} $year^{-1}$). Changes in Ca and Mn mass fractions were significantly correlated with total accumulated N. A recent meta-analysis by Mao et al. (2020) largely confirmed these patterns with significant increases for N and decreases for P, Ca, and Mg mass fractions. There was a notable difference in Mn, where the authors reported no significant effect. Subdivision of their data indicated that N addition increased Mn mass fraction in tropical forests (+26%), and had a marginally significant negative effect in temperate forests (−15%). These data suggest that tropical forests suffer less from disturbance of the C cycle under N addition, but raise questions whether Mn leaching, due to N addition-driven increases in acidity, causes reductions in Mn availability.

A synthesis by Li et al. (2015) indicated that N deposition increased fine-root N mass fractions (+18%) while reducing the C:N ratio (−14%). The changes in the chemical composition of fine roots resulted in a lower decomposition rate, an effect that was not quantified. Changes in root litter quality are also driven by changes in the abundance and species composition of (ecto-)mycorrhizal fungi on fine roots, but the available data do not allow an assessment of the latter effect. Changes in C chemistry under the influence of N enrichment have been proposed, although the evidence is not straightforward. A meta-analysis by Liu et al. (2016) suggests that N addition increases both protein (+26%) and lignin (+7%) mass fractions in plant tissues and in fresh litter. For trees the effect of N addition on protein mass fraction was significantly positive, but the effect on lignin mass fraction was marginally significantly negative. Maaroufi et al. (2016) on the other hand did not observe changes in the mass fractions of hemicelluloses, cellulose and lignin in conifer stands subjected to N addition.

Studies have provided evidence that the direct effect of N deposition (through modification of soil properties) has a larger impact than the indirect effect (through changes in litter quality), but it is evident that both pathways reinforce each other (Van Diepen et al., 2015).

3. Impacts of nitrogen deposition on soil microbiota and fauna

3.1 Changes in abundance, (functional) diversity or species composition of microbiota

A first meta-analysis by Treseder (2008), based on 14 field studies in boreal and temperate forests, showed a decline of around 20% in microbial biomass due to N addition. The negative effect was larger with higher N loading and in studies of a longer duration. Curiously, studies in which bacteria and fungi were assessed separately rather than as aggregated microbial biomass, did not show a significant negative effect of N enrichment. More recently, Zhou et al. (2017) synthesized responses of soil microbial communities to N enrichment based on 134 papers that included 454 experiments. Their data set included mostly forests (temperate to tropical) and grasslands, with only a small number of experiments in boreal forests and deserts. Averaged over all experiments, N addition reduced microbial biomass C (−6%) and increased microbial biomass N (+3%). For forests Zhou et al. (2017) noted the same effects for boreal and temperate forests, with a decline in microbial biomass C and an increase in microbial biomass N. However, for (sub-)tropical forests, N addition increased both microbial biomass C and N. The negative effects of N deposition on microbial biomass were larger in studies with a longer duration. The analysis showed significant, nonlinear relationships between microbes and N addition levels. At lower N addition (less than 100 kg N ha^{-1} $year^{-1}$) effects on microbial biomass C were positive, whereas at higher additions (between 100 and 200 kg N ha^{-1} $year^{-1}$) effects were negative. Microbial biomass N increased more strongly than microbial biomass C at lower addition levels (less than 100 kg N ha^{-1} $year^{-1}$), and did not show consistent patterns at higher additions. The microbial biomass C:N ratio declined significantly at addition levels more than 50 kg N ha^{-1} $year^{-1}$. While N addition resulted in significant acidification, regression analyses showed that the changes in microbial communities were correlated with increases in N availability, not with decreases in pH.

A recent meta-analysis by Sun et al. (2020) reported for forests (aggregated over all forest types) a negative effect of N enrichment on microbial biomass C and no effect on microbial biomass N. The authors reported that effect sizes for forests were similar to those for grasslands and croplands, and reported an average effect size for all studies (183 studies with 827 paired observations) of −12% for microbial biomass C and no significant effect for microbial biomass N, a slightly stronger effect on microbial biomass C than reported by Zhou et al. (2017). The meta-analysis by Jia et al. (2020) on N addition effects on microbial communities in forests indicated significant declines in both microbial biomass C and N. Aggregated over all ecosystems, the analysis showed significantly larger negative effects on microbial biomass C in studies of longer duration (four or more years) and in studies with stronger N loading, and variable results for microbial biomass N and C:N ratio. It is likely that the selection of papers for inclusion in these three meta-analyses is responsible for (relatively) small differences between these studies. All studies agree that N addition significantly reduces microbial biomass C.

In the meta-analysis by Zhou et al. (2017), however, the use of fatty acids (PLFAs) as a marker of microbial biomass showed no significant change after N addition. The soil microbial community can be divided in guilds that consist of fast-growing, r-selected microbes with a preference for labile materials and slow-growing, K-selected microbes that are able to grow on recalcitrant materials (Moorhead and Sinsabaugh, 2006). These groups thus differ profoundly in their influence on soil C cycling. Changes in these guilds are visible through changes in the ratio between fungi and bacteria (F:B ratio) or the ratio of Gram-positive and Gram-negative bacteria (G+:G− ratio). Among microbial groups, fungi (excluding arbuscular mycorrhizal fungi) declined significantly (−6%) whereas bacteria significantly increased (+6%), resulting in a distinct lowering of the F:B ratio. Among the bacteria, the Gram-positive bacteria responded positively (+16%), whereas the Gram-negative bacteria were unaffected (0%), resulting in a higher G+:G− ratio. The F:B ratio was more strongly negatively affected by higher N enrichment levels, whereas the opposite pattern was exhibited for the G+:G− ratio. As fungi are generally considered to be better able to decompose recalcitrant plant materials like lignin, a decrease in F:B ratio with N loading suggests reduced ability to degrade lignin, which results in a reduced litter decomposition rate and thereby SOC accumulation. Generally Gram-negative bacteria are considered to be more opportunistic bacteria that use more labile C sources than Gram-positive bacteria with a higher ability to use more recalcitrant C compounds (Fanin et al., 2019; Schimel et al., 2007; Zechmeister-Boltenstern et al., 2015). However, Ho et al. (2017) noted that this division in Gram-positive and Gram-negative bacteria is likely too crude to capture changes in bacterial community composition along environmental gradients. Zhang et al. (2018a) also analyzed a global data set with 1408 paired observations from 151 studies. They noted a significant decline with N enrichment in total microbial biomass (−13%), bacterial biomass (−17%), and fungal biomass (−19%), resulting in a marginally significant decline in the F:B ratio, whereas the G+:G− ratio remained unchanged. The microbial biomass C:N ratio declined significantly. Effects were larger with higher N application and in experiments with a longer duration. Their paper did not allow ecosystem type-specific evaluation. Jia et al. (2020) noted, for forests, no significant decline in biomass of PFLAs as marker for microbial biomass, and a decline of bacteria, but no effects on fungi and the F:B ratio.

A meta-analysis by Wang et al. (2018) showed that N addition in forests resulted in a significant decrease in species richness of bacteria, while no data were available for fungi. Diversity of fungi was negatively affected by N addition, whereas no significant change was reported for bacteria. The authors assessed the impact of N addition on bacterial and fungal groups, aggregated over all ecosystem types. In most cases the responses were variable and not significant. Only the relative abundance of Acidobacteria and Nitrospirae significantly declined after N addition, whereas the changes in eight other bacterial orders were not significant. In a forest subjected to chronic N deposition there was no effect of N enrichment on Acidobacteria, but on lower taxonomic levels changes in species composition were noted (Turlapati et al., 2013). Similarly N addition did not affect the relative abundance of Ascomycota and Basidiomycota, but the very large confidence intervals indicate that the generality of this conclusion is still uncertain (Wang et al., 2018). Nitrogen addition decreased the abundance of Agaricales (Basidiomycota), consisting of both ectomycorrhizal and ligninolytic saprotrophic fungi, resulting in a slowing down of lignin breakdown (Argiroff et al., 2019). Wang et al. (2018) also noted a highly significant relationship between the changes in microbial diversity and microbial biomass C, implying that N addition likely impacts on ecosystem function. Not many studies have been executed to assess how N deposition modifies the structure of bacterial communities. Freedman and Zak (2015) observed declines in bacterial species richness. The bacterial communities exhibited a more clustered co-occurrence network topology, implying increased biotic interactions within that bacterial community. However, as the bacterial metagenome did not exhibit modified properties, it is likely that there is sufficient physiological redundancy in bacterial functioning after N enrichment.

Changes in species richness and species diversity of bacteria and fungi after N addition were analyzed by Yang et al. (2020). In their study, N addition significantly decreased bacterial and fungal species richness in forests, but diversity of both groups was significantly increased, likely caused by vastly increased evenness, a hypothesis not further discussed by these authors. For both species richness and diversity of bacteria and fungi, the effect was strongest in the short term, and attenuated after 10 years, suggesting that the experimental treatments caused a strong initial disturbance. Variation partitioning indicated that pH and SOC content were major soil properties that affected species richness and diversity. The analysis finally showed that for effects on fungal species richness and diversity the method of assessment was important, as 454 pyrosequencing and Illumina sequencing yielded different effects. Meta-analyses such as by Yang et al. (2020) should therefore be interpreted with caution.

Many studies on species richness of microbial communities after N enrichment are based on sequencing of the ribosomal genes, but this method has the disadvantage that it also amplifies nonactive microbes. A better, but less often used method, is therefore sequencing of RNA as an estimate of the active microbial community. Freedman et al. (2015) noted that, based on DNA sequences, N addition did not reduce bacterial or fungal species richness. With RNA methods, however, fungal species richness was significantly reduced (−8%), while no effect on bacterial species richness was noted.

Fruitbodies of saprotrophic Basidiomycota can also be used as indicator for N enrichment. Simmel et al. (2017) proposed indicator values for N for these fungi. Changes in the abundance of saprotrophic Basidiomycota is correlated with their classification as nitrophobic or nitrotolerant (Kuyper, 2013). Evidence of high sensitivity of ligninolytic fungi to N enrichment was provided by Entwistle et al. (2018a). Their observations provide an explanation that with N enrichment, breakdown of lignin and lignin-like substances is hampered, as a consequence of which SOC accumulates. An early study by Morrison et al. (2016) demonstrated that chronic N enrichment stimulated the abundance and diversity of saprotrophic Ascomycota, a group of fungi with lower ligninolytic capabilities than Basidiomycota. Morrison et al. (2018) subsequently noted that in a temperate forest that was exposed to more than two decades of N enrichment, six saprotrophic fungal species of unknown taxonomic affiliation significantly declined due to N enrichment and that their decline was correlated with a strong decline in oxidative-enzyme activity, involved in the degradation of lignin. At the same time saprotrophic fungi with a lower ligninolytic potential were favored. Selection of fungi with a lower capacity for degradation of complex C compounds as a consequence of N enrichment was also reported by Treseder et al. (2021).

Next to changes in the saprotrophic fungal community, N enrichment has even larger negative impacts on the ectomycorrhizal fungal community (Lilleskov et al., 2019, 2023). Such changes, and especially those in the nitrophobic genus *Cortinarius*, have direct impacts on C cycling. Lindahl et al. (2021) showed that the presence of *C. acutus* was associated with patches with significantly lower C stocks, and suggested that its their decline therefore favors C accumulation. The decline in Basidiomycota and especially the order Agaricales after N enrichment, as reported by Entwistle et al. (2013) could have been due to either a decline in ectomycorrhizal or saprotrophic fungi.

Meta-analyses can demonstrate generalized effects of N enrichment on soil microbial communities (but see Section 7 for a critical reflection on the use of meta-analysis), but due to limited data availability, they can currently not determine whether these changes are primarily driven by changes in soil properties or by changes in litter quality. It is therefore strongly recommended that studies on the effects of N deposition provide data on both changes in soil properties and changes in litter properties (Vicca et al., 2018). Maaroufi et al. (2017) demonstrated that litter decomposition rates were reduced independent of litter origin, when litter was placed in high-N plots compared to low-N plots, thus demonstrating the dominant role of changes in soil processes due to N enrichment. Correlations between decomposition rates and microbial groups (fungi, Gram-negative bacteria) showed that N addition changed microbial communities (composition, biomass) that resulted in lower C dynamics, a result that was similar to that obtained by Treseder (2008). Data by Hobbie et al. (2012) on the other hand demonstrated an initial role for both litter origin and external N supply in enhancing initial decomposition rates, but only a role for external supply on the final undecomposable fraction.

3.2 Changes in species composition, abundance or functional diversity of soil fauna

As a consequence of N-induced changes (declines) in microbial biomass, soil fauna (especially nematodes, microarthropods like mites and springtails, and earthworms), that feeds on those microbes, will likely be negatively affected. Shaw et al. (2018) reported an increased nematode abundance, but reduced species diversity due to N enrichment in a subalpine coniferous forest. They reported a shift toward bacterivorous nematodes, implying a decline in the F:B ratio. Gan et al. (2013) studied the effects of N addition on microarthropod abundance in temperate broad-leaved forests. N enrichment reduced the abundance of microarthropods with almost 50%, possibly because of the reduced energy that enters the saprotrophic soil food web when decomposition is retarded. There were species-specific responses to N deposition in oribatid mites. However, there were no effects of N enrichment on species richness. The decline of microarthropods coincided with a reduced decomposition of confined leaf litter, although an additional role of soil fauna in reducing litter decomposition could not be determined. The scarce literature in that regard is not consistent. Zhang et al. (2016a) noted that N addition and exclusion of soil fauna both reduced litter decomposition, but there was no interaction between both factors. A recent study by Liu et al. (2021) on the other hand reported a significant interaction between soil mesofauna and N addition on decomposition rate. More research seems required to understand the extent to which N deposition driven changes in microbial abundance and composition cascade through the saprotrophic food web.

4. Effects of nitrogen deposition on enzymatic activities

In this section we deal with five classes of enzymes, viz. hydrolytic enzymes (e.g., cellulases), oxidative enzymes (e.g., peroxidases), chitinases and amino peptidases (two classes of enzymes associated with the acquisition of N from either microbial necromass or proteins), and phosphatases (enzymes associated with the acquisition of phosphorus from organic sources).

The rationale for separating hydrolytic and oxidative enzymes, both of which are involved in C cycling, is the fact that the first class of enzymes liberates or degrades C compounds that are associated with high microbial carbon use efficiency (CUE), and where the C is used for both catabolic and anabolic purposes. Oxidative enzymes on the other hand are associated with low CUE, and as a result the oxidation of these compounds is only associated with catabolic processes. It is often stated that oxidation of lignin does not generate energy and that very little C (at best) is used for the production of microbial biomass (Margida et al., 2020; Moorhead et al., 2013a). The main benefits of oxidative activity are that it either liberates other C compounds (cellulose is largely inaccessible to microbial attack due to its strong protective association with lignin) that then is used for the build-up of microbial biomass or liberates organic N and P, a process especially relevant for soil microbes that are nutrient-limited rather than C-limited (energy-limited) such as mycorrhizal fungi (Kuyper, 2017; Lindahl and Tunlid, 2015). These contrasting effects of oxidative activity, viz. enhanced C access for saprotrophs and enhanced N access through mining for ectomycorrhizal fungi, explains what Bonner et al. (2019) considered a counterintuitive result of N enrichment. In their model, alleviation of N limitation after N addition resulted in enhanced rather than reduced oxidative-enzyme production. Bonner et al. (2019) as an alternative suggested that N shifts the balance between white-rot fungi, who produce peroxidase, and brown-rot fungi, who produce iron reductases and hydrogen peroxide. However, the fact that upregulation of this nonenzymatic mechanism of lignin degradation by brown-rot fungi occurred only in one season, makes the hypothesis weakly supported. The major role of oxidative activity for N-mining by ectomycorrhizal fungi is consistent with the differential effects of N deposition on C dynamics in ectomycorrhizal and arbuscular mycorrhizal forests.

Recognizing two classes of enzymes associated with the organic N cycle is relevant in the light of the view of changing perspectives of the nature of soil organic matter, where plant necromass is increasingly considered as less important than microbial necromass (Hoffland et al., 2020). Next to changes in individual enzymes, stoichiometric considerations come into play, as it is the balance between the acquisition of C, N, and P that determines microbial fitness (Moorhead et al., 2012). In stoichiometric approaches both the ratio between hydrolytic and oxidative enzymes, and the ratios between enzymes involved in the acquisition of C (restricted to hydrolytic enzymes), N and P are considered. One of the first examples of such a stoichiometric approach to enzyme activity was provided by Sinsabaugh et al. (2002) who noted that N enrichment shifted enzyme activity from oxidative to hydrolytic activity, and away from N acquisition toward P acquisition of organic sources.

Studies that went at a deeper level of understanding enzymatic properties, through assessment of N enrichment effects on Michaelis–Menten properties (V_{max} and K_m), have not been reviewed as there is still too little information available to allow generalization.

4.1 Changes in oxidative enzymes

Reduced activity of phenol oxidase and ligninolytic activity after N addition was first described by Carreiro et al. (2000). Based on a data set of 40 studies Chen et al. (2018b) observed that N addition resulted in a significant decrease of oxidative enzymes (lignin-modifying enzymes, including peroxidase, phenol oxidase, and polyphenol oxidase), and increase in hydrolytic enzymes (cellulases) that went together with an increase in SOC stocks. Jian et al. (2016), based on 65 studies, reported similar outcomes, i.e., an increase in hydrolytic enzymes and a decrease of oxidative enzymes. A subdivision of their dataset for various ecosystem types (forest, grassland, shrubland, farmland) showed a significant increase in hydrolytic enzymes and a significant decrease in oxidative enzymes due to N addition in forests. Chen et al. (2018b) also observed a highly significant negative correlation between the amount of oxidative enzymes on the one hand and C accumulation on the other, explaining 40% of the variation in N addition effects on C stocks. The reduction of oxidative enzymes had a major effect on the recalcitrant SOC pool. Sinsabaugh (2010) and Sinsabaugh et al. (2005) earlier described the linkages between N enrichment, reduction of phenol oxidase and peroxidase, and accumulation of SOC.

Chen et al. (2018b) noted that N addition, by reducing the production of oxidative enzymes and/or by increasing the production of hydrolytic enzymes, aggravated microbial C limitation. Both processes result in a faster decline of substrate quality under N addition. The faster decline in substrate quality drives a decline in CUE by microbes, resulting in a lower microbial biomass and especially of (basidiomycete) fungal biomass, the groups with the higher capacity to produce oxidative enzymes.

While it is unknown to what extent oxidative activity in forest ecosystems is due to ectomycorrhizal and saprotrophic fungal activity, it is likely that both lignin modification and degradation (to increase access to shielded cellulose) and modification and degradation of humic substances (to increase access to nutrients in organic forms) are relevant. A possible major role for ectomycorrhizal fungi is suggested by observations that N addition has stronger negative effects in (ectomycorrhizal) forests than in grasslands that are dominated by arbuscular mycorrhizal fungi, fungi that do not produce oxidative enzymes (Sinsabaugh, 2010). The observations by Lindahl et al. (2021) about a positive correlation between the abundance of the ectomycorrhizal fungal species *Cortinarius acutus* and low SOC pools, and the nitrophobic character of *Cortinarius* species, is consistent with a major ectomycorrhizal role. Similar results were reported by Argiroff et al. (2022), who noted significant negative correlations between abundance of ectomycorrhizal fungi with peroxidase activity, and soil inorganic N availability, lignin-derived soil C, and SOC. Waldrop et al. (2004) and Waldrop and Zak (2006) found that N addition decreased oxidative enzymes and increased the SOC in forests dominated by ectomycorrhizal trees, but increased phenol oxidase and reduced SOC stocks in stands dominated by arbuscular mycorrhizal trees. Their data thus point to the importance of mycorrhizal status when assessing effects on N enrichment on soil C fluxes (Suz et al., 2021).

Some peroxidases need Mn as cofactor, and positive correlations between Mn availability and litter decomposition have been established (Stendahl et al., 2017; Whalen et al., 2018). Whalen et al. (2018) reported a strong correlation between Mn availability and the activity of peroxidase and phenol oxidase, implying that N-induced reductions in Mn availability are a major driver for reduced oxidative activity under N enrichment. However, it is not yet well known whether and how N enrichment and the resulting acidification (Tian and Niu, 2015) modify the availability of Mn. The roles of Mn in both stabilizing and destabilizing SOC demand further study (Li et al., 2021).

4.2 Changes in hydrolytic enzymes

A meta-analysis based on 62 publications indicated that four hydrolytic enzymes (α-1,4-glucosidase, +20%; β-1,4-glucosidase, +11%; β-ᴅ-cellobiosidase, +22%; and β-1,4-xylosidase, +13%) were stimulated by N addition (Chen et al., 2017), resulting in an average increase of 13% in hydrolytic-enzyme activity. For each of these enzymes there was a positive correlation between fractional increase in enzyme activity and fractional increase in soil respiration. However, the positive effect on enzyme activity was around 2.5 times larger than the effect on soil respiration, indicating that changes in other enzymes have to be considered as well. The data showed that the beneficial effect increased over time and with N dose. Their analysis included all forms of N enrichment and showed that organic N sources have a larger effect than mineral N sources. Analysis of the data of mineral-N addition showed a significantly positive effect of addition of NH_4NO_3, whereas the effects of both ammonium and nitrate were not statistically significant, an effect that was not explained. Restriction of the dataset to forests showed a smaller stimulation of enzyme activity of 6% possibly due a lower representation of studies that included application of inorganic N.

Taken together these meta-analyses show a shift, under the influence of N addition, away from oxidative activities and toward hydrolytic activities, consistent with the original report by Sinsabaugh et al. (2002). This shift initially enhances but later retards litter decomposition, as the opposite effects of both classes of enzymes result in a decoupling of the degradation of lignin and cellulose, and as a consequence a faster decline of litter quality and a lower limit value.

4.3 Changes in enzymes associated with nitrogen acquisition from organic sources

Enrichment with mineral N likely decreases microbial investment in enzymes associated with the acquisition of N from organic sources. Changes in activities of enzymes that are involved in N acquisition from proteins, such as leucine aminopeptidase or glycine aminopeptidase, and enzymes involved in N acquisition from microbial necromass, such as chitinase (N-acetyl glucosaminidase), are therefore likely indicators of effects of N enrichment on microbial abundance and activity.

A meta-analysis by Chen et al. (2018b) indicated that N addition in forests does not have an effect on the activity of chitinase and leucine aminopeptidase, whereas glycine aminopeptidase showed a reduction in activity. The meta-analysis by Jian et al. (2016) did not show significant changes in both classes of enzymes associated with N acquisition. Frey et al. (2014) did not assay these enzymes directly, but determined proteolytic activity by measuring increase in amino acids after incubation. Their study showed a significant decline in proteolytic activity (47% −60%) with N addition. Lucas and Casper (2008) on the other hand observed that N addition reduced oxidative enzymes (as predicted), but increased proteolytic activity, resulting in a significantly negative relationship between phenol oxidase activity and proteolysis. It is not clear why both studies contrast with the results of both meta-analyses. Possibly the extent to which oxidative and proteolytic enzymes are produced by ectomycorrhizal and saprotrophic fungi is part of the explanation.

4.4 Changes in enzymes associated with phosphorus acquisition from organic sources

Increased availability of mineral N due to N enrichment likely results in an unbalanced acquisition of N and P, and this results in a relative upregulation of phosphatases. Two meta-analyses have been published on this topic. Jian et al. (2016) reported an increase of +11% under the influence of N addition, which seems much lower than an earlier meta-analysis by Marklein and Houlton (2012) who reported an increase of +46%. However, the latter data refer to untransformed data, while log-transformed data, in order to comply with the demand of symmetry around the mean, indicated an increase of 11%, being equal to results of Jian et al. (2016). Consistent with this hypothesis of a shift away from N-limitation toward P-limitation, Zhang et al. (2018b) reported an increase in phosphomonoesterase activity as a consequence of N additions and suggested that due to this upregulation P limitation is alleviated. The effect occurred both on P-poor and P-rich soils. That conclusion was reinforced by a meta-analysis by Chen et al. (2020), based on 140 studies with 668 observations, showing that N addition alleviates P limitation as phosphatase activity increased with 13%. Restriction of the analysis to forest sites confirmed this result. However, the effect is likely to be a short-lived response, as studies lasting less than 5 years showed a significant increase in phosphatase activity (+28%), whereas long-term N addition did not exhibit changes compared to ambient conditions. The implication of this observation is still under debate. Under the assumption that phosphatase activity is a proxy for P-limitation, Chen and Moorhead (2022) suggested that these data imply mitigation of P-limitation over time. However, an alternative explanation would be that long-term N deposition inhibits microbial activity and that this negative effect overrides any enzymatic upregulation, and that mitigation of P-limitation in the longer term is unlikely. Ma et al. (2021) confirmed the general positive response of N enrichment on phosphatase production (+8%) and a very strong difference between short-term studies (lasting 3 years or less) and studies lasting 3−10 years (+49% and −11% respectively). Separation of AM and EcM forests showed that for AM forests there was a positive effect of N enrichment on phosphatase activity (+28%), whereas for EcM forests there was hardly any effect (+4%, not significantly different from zero). Unfortunately their Fig. 8 provides an incorrect image, as it depicts a stronger positive response in the case of EcM trees than of AM trees. For a heathland ecosystem, Kritzler and Johnson (2010) reported increased phosphatase activity in response to higher levels of N deposition, as a consequence of which P acquisition was increased in the ericoid mycorrhizal *Calluna vulgaris*, but this effect was not noted for the arbuscular mycorrhizal grass *Dactylis glomerata*. Whether alleviation of P limitation impacts C cycling is currently unknown (Luo et al., 2022).

4.5 Changes in enzymatic stoichiometry

A simple way to address interactions between enzyme activities under the influence of N addition is to assess changes in enzyme ratios. The above treatment of the various enzymes indicates that the balance of oxidative and hydrolytic enzymes shifts in the direction of the latter class, resulting in more rapid declines in litter quality and hence a lower limit value, the amount of litter than can be decomposed, so a lower limit value translates into a larger SOC pool. Similarly it is likely that N addition shifts the ratio of enzymes involved in the acquisition of N and P of organic sources, a mechanism through which microbes delay or alleviate P limitation. Sinsabaugh et al. (2009) additionally proposed to use the ratio of hydrolytic enzymes and organic-N acquisition enzymes and the ratio of hydrolytic enzymes and organic-P acquisition enzymes. A more formal approach to enzymatic stoichiometry has been proposed by Moorhead et al. (2013b, 2016). Because oxidative enzymes do play only a very limited role in the acquisition of C for biomass build up, as the CUE of such compounds is very low, most stoichiometric models do not include oxidative enzymes. However, because oxidation of recalcitrant, humic substances is a prerequisite step for the activities of enzymes associated with the acquisition of N and P from organic sources, correlations between oxidative enzymes and organic-N and organic-P acquisition enzymes can be expected. Sinsabaugh and Shah (2011) indeed noted positive correlations between oxidative enzymes and organic-N and organic-P acquisition enzymes, whereas no relationship existed between oxidative and hydrolytic enzymes. A formal meta-analysis of N deposition effects on enzymatic stoichiometry was published by Chen et al. (2018b). Their meta-analysis, based on 36 studies, showed that N addition did not change the ratio of C, N, and P acquisition and did not alleviate P-limitation, but provided evidence for increased C-limitation. As the number of studies in forest ecosystems is very limited, these results should be interpreted with caution.

4.6 Changes in functional genes

Apart from the use of next-generation sequencing to assess the taxonomic composition of microbial communities under the influence of N addition (discussed above), several studies have assessed N addition impacts on functional genes, either on DNA level or RNA level, which indicates the actual expression of those genes. Entwistle et al. (2018b) demonstrated that two decades of N deposition modified the composition of fungal peroxidase genes. This modification occurred simultaneously with lower decomposition rates and increased accumulation of SOC. Zak et al. (2019) reported substantial downregulation of genes for peroxidases (a reduction with 60%–80%), which went together with a reduction in peroxidase enzyme activity, but without changing fungal species composition. As peroxidases seem more sensitive than laccases to N enrichment (Hassett et al., 2009; Hofmockel et al., 2007), it is possible that soil microbial communities provide some resistance against N enrichment. Shifts in laccase gene sequences, but without decline in their relative abundance, under the influence of N fertilization have been recorded by Lauber et al. (2009). Hesse et al. (2015) assessed transcript sequences of carbohydrate-active enzymes (CAZymes), and noted changes as a consequence of N deposition, partly through overexpression of bacterial CAZymes, partly through suppression of fungal CAZymes related to lignocellulose degradation. Whereas gene abundance of fungal oxidases decreased as a consequence of N enrichment, gene abundance of bacterial laccase-like multicopper oxidases increased, which at least partly compensates for the losses of oxidative capabilities of microbial communities under N enrichment (Freedman and Zak, 2014). Moore et al. (2021) observed that N enrichment increased fungal functional genes for hydrolytic enzymes and reduced fungal functional genes for oxidative enzymes, parallel to a shift in enzyme activity, however Eisenlord et al. (2013) reported similar decreases −15%) in the richness and diversity of genes coding for hydrolytic and oxidative enzymes.

5. Effects of nitrogen deposition on litter decomposition and soil respiration

5.1 Changes in litter decomposition

Most studies on litter decomposition pertain to leaf and needle litter decomposition. Studies on wood and on root decomposition are underrepresented. This underrepresentation is especially relevant for fine-root decomposition, as the belowground inputs constitute the major fraction of SOC (Basile-Doelsch et al., 2020; Jackson et al., 2017; Rasse et al., 2005) both as a consequence of lower decomposability or higher recalcitrance (Xia et al., 2015) and of more intimate associations with the mineral-soil matrix. Jackson et al. (2017) found large differences between root and shoot decomposition rates for agricultural crops. Data for fine-root and leaf and needle litter decomposition showed smaller differences (Kou et al., 2015), although fine roots still decomposed at a lower rate than leaf and needle litter.

Various meta-analyses about weight loss of confined litter under the influence of N enrichment have been published by Knorr et al. (2005) and Zhang et al. (2018b). The meta-analysis by Knorr et al. (2005), based on 24 studies with 500 observations, summarized studies that investigated the effect of N enrichment on litter decomposition. Their studies included not only forests but also grasslands and tundra ecosystems. They noted a large range in responses, ranging from a 64% increase in decomposition rate to a 38% decrease. Because of the large variation between studies there was no overall significant effect of N enrichment on litter decomposition. Subdivision of the data set indicated that studies that lasted less than 24 months reported a significant increase in decomposition rate of 7%, while studies lasting more than 24 months reported a significant decline of 18%. They noted that the negative effect of N addition was stronger for litter of lower quality, usually litters with higher lignin mass fractions and/or higher C:N ratios. By averaging over studies, meta-analysis make context-dependent impacts difficult to evaluate, as shown by a recent meta-analysis of N deposition and decomposition, based on 55 studies with 3434 paired observations

(Zhang et al., 2018). The authors noted no significant effect of N enrichment on litter mass loss. However, their data showed a significant correlation between the decline in decomposition rate and the duration of the study. The correlation between the amounts of N added and the change in decomposition rate was not significant. However, translating these outcomes to impacts of N deposition on litter decomposition in forests is difficult, considering the vary large range in application rates, ranging from 2 to 640 kg N ha^{-1} $year^{-1}$ (average 123 kg N ha^{-1} $year^{-1}$), rates that are well above N deposition in forests.

While some studies have pointed out similarities in N enrichment effects on litter decomposition in forests and grasslands, a meta-analysis from China (Su et al., 2021) indicated positive effects of N addition on litter decomposition in grasslands (+4%), but negative effects in forests (−15%). A subsequent meta-analysis by Su et al. (2022) confirmed positive effects in grasslands (+4%) and much smaller negative effects in forests (−1%) than in Su et al. (2021), a difference that was not explained.

Exceptions to these patterns have been reported. Vivanco and Austin (2011) observed strong positive effects of N addition on litter decomposition of species of *Nothofagus* and reported that after 24 months, decomposition of lignin-richer litters were more stimulated by N additions than litters that were lower in lignin mass fraction. They therefore suggested that N enrichment in unpolluted areas as in southern South America shows divergent responses compared to forests in the industrialized areas in the global north. A possible explanation is that chronic N deposition is fundamentally different from a rapid addition of N fertilizer, even though the authors used urea as a slow-release fertilizer in order to mimic chronic N deposition. A further possibility for the discrepancy is that their study lasted 24 months, a period for which also Knorr et al. (2005) reported stimulation of litter decomposition. A meta-analysis by Bejarano-Castillo et al. (2015) on N enrichment effects on the C cycle in tropical forests indicated large differences between different ecosystems. Decomposition was significantly enhanced in montane rainforests, which are assumed to be more frequently N limited, but reduced in lowland forests, where P or base cation limitation is more likely.

Positive effects of N addition on the initial stages of litter decomposition are generally explained through a lowering of the litter C:N ratio. These observations are supported by general theories of litter decomposition, where in the initial decay stage saprotrophic organisms and hence saprotrophic activity (decomposition) are nutrient-limited, and more specifically N-limited, whereas in later stages saprotrophic organisms and activity are energy-limited, often referred to as C limited. Enzymatic data (see above) are consistent with this mechanism. Chemical analysis of both aboveground and fine-root litter that decomposed under ambient and elevated N levels further supports the indication that the negative long-term effect of N addition is due to less efficient degradation of lignin-like or acid-unhydrolyzable materials (Xia et al., 2017).

Whittinghill et al. (2012) looked into more detail how N addition affects decomposition rates. They subdivided the decomposition process in three stages, i.e., an initial stage in which decomposition is limited by nutrients, especially N (or P, in tropical forests, see Cui et al., 2021), a second phase where decomposition is regulated by the decay of lignin and the lignified carbohydrates, and a third phase of very slow decomposition that can be approximated by a constant fraction of remaining material. The amount of litter that is decomposed when this asymptote is reached, is known as the limit value (Berg, 2000). Their analysis showed no effects of external N on the first phase of decomposition. In the second phase an increase of cellulose decomposition (+9%) and a decrease of lignin decomposition (−30%) was shown, but the decomposition rate of the litter was not affected by N. In the final phase they authors noted a larger undecomposable fraction as evidenced by a lower limit value from around 70%−60%. Berg (2000) noted that the limit value in pine forests with ambient N levels was on average around 80%, and in N-fertilized stands around 70%, providing support for the suggestion that the effect of N addition is primarily due to a lowering of the limit value.

However, the model used by Whittinghill et al. (2012) and the underlying concept of limit value is problematic. The concept of an asymptote, a limiting value, was introduced by Howard and Howard (1974) in their attempts to fit decomposition data. The authors noted that weight loss of confined litter indicates the ability of microbes to decompose litter in the absence of soil fauna, and that limit values therefore underestimate the amount of litter than can be decomposed. Changes in the relative decomposition rate of lignin and carbohydrates like cellulose, as happens as a consequence of N deposition, modify the curvature of graphs that plot mass remaining against time, and hence mathematically, but not necessarily biologically, change in the asymptote, that is the limit value. Furthermore, modeling litter decay as separate stages with a residual undecomposable fraction overlooks interactions between litter components, for instance priming of SOC decomposition under the influence of exudates. It is therefore important to evaluate the effects of N addition on litter decomposition in models that treat litter decay as a continuum, like the one proposed by Yang and Janssen (2000).

A meta-analysis of the effects of N addition on fine-root decomposition was published by Fu et al. (2022), based on 20 studies, mostly from China, with 123 independent data points. The analysis showed a general retardation of 20%, with a slightly larger retardation for broad-leaf fine roots than for conifer fine roots. The negative effect of N addition increased over time and increased with root lignin concentrations. Lignin concentration was the best predictor for N effects on modifying fine-root decomposition. The authors noted a larger slowing down of fine-root decomposition when oxidative-enzyme activity by phenol oxidases was more negatively affected by N deposition. Xia et al. (2017, 2018) reported faster decay of fine roots of sugar maple under N addition than under ambient conditions after 1 year, but a very significant slowing down afterward. After 3 years the decomposition constant under N addition was 15% lower than under ambient conditions. Both lower mass loss and an enhanced interaction of remaining root material with the mineral matrix (Zak et al., 2017) are major drivers for SOC accumulation under N enrichment.

Due to N deposition, the abundance and species composition of mycorrhizal fungi, especially of ectomycorrhizal fungi, change (Lilleskov et al., 2023). The literature on decomposability of ectomycorrhizas, compared to nonmycorrhizal roots is inconsistent, with both claims that ectomycorrhizas are more (Langley et al., 2006) and less (Koide et al., 2011) recalcitrant than nonmycorrhizal fine roots. Whether N deposition differentially affects decomposability of ectomycorrhizal and arbuscular-mycorrhizal fine roots is currently unknown.

Decomposition of wood, a substrate with a very high initial C:N ratio, is likely limited by N availability. Cord-forming Basidiomycetes have the ability to import N into decaying wood through their extensive mycelial networks that scavenge the soil. Under such conditions N addition could enhance the initial decomposition of wood blocks. This hypothesis was confirmed by Bebber et al. (2011) for two species of wood decomposer fungi colonizing blocks of beech wood.

5.2 Changes in soil respiration

Soil respiration, the flux of CO_2 from the soil to the atmosphere, is a major indicator of soil C dynamics. Soil respiration consists of two fluxes, called autotrophic respiration, CO_2 production by living roots, associated mycorrhizal fungi, and from exudates by roots and mycorrhizal fungi; and heterotrophic respiration, CO_2 production due to the breakdown of SOC. Several methods exist to separate both fluxes (Halbritter et al., 2020; Hanson et al., 2000; Ryan and Law, 2005), but different methods potentially lead to divergent results (Zhou et al., 2014). Root exclusion methods seem to overestimate the (negative) effects of N enrichment on heterotrophic respiration and hence underestimate effects on autotrophic respiration compared to isotopic methods. This is possibly so because interactions between the drivers of both fluxes, through priming of SOC decomposition and through ectomycorrhizal fungal activity on oxidation of SOC, are not accounted for. The small number of studies, combined with substantial variation within certain biomes makes further investigation of potential methodological biases imperative.

In a first meta-analysis restricted to forest ecosystems based on 20 N manipulation experiments, Janssens et al. (2010) reported that under the influence of N deposition both aboveground and belowground C inputs did not change, whereas the soil C pool increased and microbial biomass C decreased. They furthermore noted decreases in soil respiration which was caused both by significant declines in autotrophic and heterotrophic respiration. Based on a subsequent meta-analysis of 139 studies, Zhou et al. (2014) estimated the effect of N addition on soil respiration in forests as slightly, but significantly negative (−1.4%). Split in both components, N addition did not result in significant changes in autotrophic respiration, but significantly reduced heterotrophic respiration by around 20%. Averaged over all biomes, the analysis showed that with longer periods of N enrichment the decline in soil respiration was larger. Long-term studies at the same site confirm this time dependency of effects. Burton et al. (2004) reported a positive effect of N enrichment on soil CO_2 efflux after 1 year, but negative effects in the subsequent fifth, sixth, and eighth years, an effect that they attributed to reduced microbial heterotrophic respiration; and by Bowden et al. (2004), who observed an increase in soil respiration the first year after N amendment but a substantial suppression (−41%) in the 13th year. Xing et al. (2022a) in a plantation of *Larix gmelinii* observed a positive effect of N addition in the first year (+8%), which became subsequently negative and showed a strong negative effect (−21%) in the 11th year. They also noted a much stronger negative effect with higher N loading and suggested that such experimental treatments, which add higher amounts than what is normally found in N deposition studies, likely overestimate the effect of N deposition on C sequestration. Average annual temperature exerted a large effect on soil respiration with cold sites exhibiting more negative responses and warmer sites exhibiting less negative or even positive responses to N enrichment (Zhou et al., 2014). Increases in soil respiration after N fertilization in a lowland tropical rainforest, as noted by Cleveland and Townsend (2006), further support the hypothesis that responses to N enrichment differ between boreal and temperate forests, which are usually N-limited, and tropical forests, which are more often P-limited. However, a meta-analysis of N-rich tropical forests indicated that N addition increased soil C stocks (Lu et al., 2021).

A major interactive effect between N addition and annual temperature was suggested through the meta-analysis by Zhong et al. (2016), where N addition had a significantly negative effect on soil respiration in boreal (−17%) and temperate forests (−7%), but no significant effects on tropical forests. Again, this differential effect could also be caused by differential nutrient limitation.

Likely due to small sample size, the effects on heterotrophic respiration were not significant in all three forest biomes, but averaged over all forest sites reductions in heterotrophic respiration were larger with higher N addition. As boreal and temperate forests are often dominated by ectomycorrhizal trees and tropical forests by arbuscular mycorrhizal trees (Read, 1991), temperature is likely partly be a proxy for differential sensitivity to N addition in forests that are dominated by different mycorrhizal symbioses. Support for this suggestion comes from the highly significant correlation between changes in microbial biomass C and changes in soil respiration. Scaling relations between both properties are not fully understood. Ramirez et al. (2012) reported for 28 different soils in North America, from a broad range of ecosystems, a decrease in heterotrophic soil respiration by 11% and a decreased microbial biomass of 35%. Such mismatches suggest that changes in microbial C use efficiency (CUE) drive differential responses between both parameters. A study by Spohn (2015) suggested that N addition does not affect microbial biomass but results in a lower respiration per unit microbial biomass. She compiled 14 studies with 48 observations on the relationship between litter C:N and CUE and noted that both parameters were significantly negatively correlated. The study did not address the extent to which a low CUE at high C:N is due to microbial N-limitation or a high CUE at low C:N is due by repression of oxidative enzymes. A theoretical approach by Ågren et al. (2001), supported by a litter decomposition study in an N-fertilized forest, suggested that both increases in decomposer CUE and more rapid formation of poorly decomposable material (driven by reduced oxidative capacity) are major causes of observed increases in microbial CUE, with a smaller role for a reduced growth rate by saprotrophic microbes. Their results are in contrast with those of Riggs and Hobbie (2016), who reported that N addition in grasslands did not increase CUE or reduce oxidative enzyme activity, but reduced microbial biomass that was considered the driving factor for C accumulation under N addition. Declines in microbial biomass under N deposition occur commonly (Treseder, 2008). However, feedbacks between changes in CUE and changes in microbial biomass are insufficiently known. It is to be expected that initial increases in CUE result in a faster decline of substrate quality, which subsequently reduce microbial biomass and likely CUE of that more recalcitrant material.

One major issue in the assessment of heterotrophic respiration should be mentioned, i.e., that respiration data are often expressed on an area basis (kg $C{-}CO_2$ ha^{-1} $year^{-1}$). If productivity and hence litter fall increase, litter accumulates and there could be higher respiration per unit area under N addition than under ambient conditions. This effect of litter and humus accumulation could counter negative effects of N enrichment on respiration per unit carbon (g $C{-}CO_2$ g^{-1} SOC $year^{-1}$) if increases in litterfall outweigh reductions in respiration rate. It can be useful to express the heterotrophic component of soil respiration rate both per unit C and per unit area.

While changes in autotrophic respiration under N deposition are not the topic of this review, it should be noted that autotrophic respiration (or rhizosphere respiration) consists of a plant component (often increased if N-limitation for plant growth is alleviated and more *absolute* root biomass is formed even though *relative* allocation to fine roots is reduced) and a mycorrhizal fungal component (where N enrichment results in a decline of mycorrhizal biomass and respiration by the mycorrhizal mycelium, which is most pronounced in the case of ectomycorrhizal fungi, see Olsson et al., 2005, Vallack et al., 2012; Lilleskov et al., 2023). Both changes in fine-root mass and ectomycorrhizal fungal biomass affect heterotrophic respiration, because of changes in exudates that prime the breakdown of SOC and changes in production of oxidative enzymes that oxidize SOC, as was reported for species of *Cortinarius* by Lindahl et al. (2021). Reduced respiration in the rhizosphere due to N fertilization, which ranged from −36% to −46% in the study by Phillips (2007), may be attributable to such interactions between mycorrhizal and saprotrophic fungi in the rhizosphere.

A subsequent meta-analysis by Zhou et al. (2016) assessed main and interactive effects of global-change factors on soil respiration and its components. In many cases combinations of N additions and other factors of global change (elevated CO_2, higher temperatures) were additive, and synergistic or antagonistic interactions were much more rare. The analysis did not allow to specifically assess the interactive effects of N and climate change in forest ecosystems.

5.3 Differential effects of nitrogen deposition on litter and soil carbon pools

While the literature has overwhelmingly reported the negative effects of N deposition on soil C fluxes, the question about differential sensitivity of various C pools has received far less attention. In this section we discuss differential sensitivities of C pools according to four contrasts: labile versus recalcitrant pools; free C pools versus mineral-protected pools; plant-derived litter versus microbial necromass and free C versus C in both micro and macroaggregates.

In the analysis on N addition effects on SOC pools, Rocci et al. (2021) showed a general SOC enrichment of 6%. The effect on particulate organic C (POC) was much larger (+13%) than the effect on mineral-associated organic C (MAOC (+4%). The effect of experimental duration was evident for POC but not for MAOC, and indicated that POC stocks increased over time, whereas MAOC stocks did not. In a predominantly arbuscular mycorrhizal forest, Eastman et al. (2022) noted a reduction of decomposition after N addition (−11%), but a larger proportion of C in the pool of particulate organic matter. However, only very few studies have addressed that question, which is crucial for estimates how N deposition would affect C sequestration, as the particulate organic carbon pool is in principle infinite, whereas the mineral-associated organic carbon pool is subject to saturation. These results imply that N deposition reduces microbial activity and biomass, resulting in both more partly undecomposed organic carbon with a larger contribution of lignin-like substances, and a smaller contribution of microbial biomass and necromass that is more easily stabilized on mineral surfaces. The data by Rocci et al. (2021) and Eastman et al. (2022) cannot easily be reconciled with the CAMP (Carbon, Acidity and Mineral Protection) model by Averill and Waring (2018) where N addition alleviates microbial N limitation, resulting in faster decomposition, declines of POC and increases of MAOC stocks, and where only N-addition driven acidification retards decomposition, increases of POC and decreases of MAOC stocks. The problem with their model seems the focus on microbial N-limitation that is alleviated by N deposition. Data by Chen et al. (2018b) and Rappe-George et al. (2017) demonstrate C-limitation, which is aggravated due to N deposition.

Several experimental studies confirm the differential response of MAOC and POC to N addition. Bowden et al. (2019) reported significant C accumulation after chronic N loading, with an 88% increase in the C pool of the organic layer and no significant changes in the mineral layer, consistent with the hypothesis that the earlier stages of decomposition are most sensitive to N deposition. The data by Rocci et al. (2021) confirm an earlier meta-analysis by Chen et al. (2018a), who noted that the light fraction (comparable to POC) was more responsive to N addition (increase of +18%) that the heavy fraction (comparable to MAOC; increase of 3%). At the same time, Chen et al. (2018a) reported that the permanganate-oxidizable (POX) fraction of SOC responded more positively to N enrichment (10%) than the unoxidizable fraction (+5%), which suggests that part of that light fraction is more sensitive to oxidative than hydrolytic enzymes. While some studies have treated the POX fraction as a labile C fraction, there is ample evidence that the POX fraction contains aromatic and humic substances that are generally not considered as labile, and that are, due to their surface charge, able to sorb to the mineral soil matrix. Further studies toward characterization of the POX and unoxidizable fraction and their sensitivity to N enrichment are clearly warranted. Lu et al. (2021) analyzed the effects of N enrichment on C in aggregates. The authors observed increased C in macroaggregates, and no effect on microaggregates, in forests, concomitant with a decrease in soil pH. It was suggested that acidification was a major driver of more C in macroaggregates.

Sensitivity of individual C compounds has also been evaluated. Consistent with the changes in enzymatic activity, N addition reduced the mass fraction of cellulose and increased the mass fraction of lignin (Hasegawa et al., 2021). Not all studies noted that effect. Thomas et al. (2012) noted for a sugar maple forest that N addition did not modify the amount of lignin or the oxidation state of lignin. However, their study did not provide evidence that N addition retarded decomposition. Studies on effects of N enrichment on lignin degradation or ligninolytic activity do not always focus on lignin only. Studies frequently refer to gravimetric lignin or Klason lignin, a pool of residual C that cannot be dissolved in strong acids. That pool contains both lignin and transformed products of both plant and microbial origin. Earlier theories on the decomposition process, with a phase of nutrient-limited decomposition where lignin is not degraded, followed by a phase where lignin regulates decomposition rate, have often been based on gravimetric lignin. However, such theories proved to be incorrect (Hall et al., 2020; Klotzbücher et al., 2011). The impact of N addition of decomposition of "true" lignin is therefore more difficult to assess and the data by Thomas et al. (2012), who used copper oxide for lignin assessment, reinforces that conclusion. A study by Thomas et al. (2013) indicated that N addition did not have any impacts on the degradation or relative oxidation state of leaf lignin of sugar maple, and it was suggested that lignin accumulation due to N addition was either due to lignin in root material and/or to lignin-like substances in microbial necromass.

Other changes in sensitivity in degradability of different C compounds turned out to be dependent on the nature of the mycorrhizal symbiosis. Gallo et al. (2005) noted that N addition reduced the decomposability of waxes and lipids in litter of ectomycorrhizal trees, but increased their decomposition in litter of arbuscular mycorrhizal trees. Aromaticity of dissolved organic C (DOC) on the other hand increased in litter of ectomycorrhizal trees and decreased in litter or arbuscular mycorrhizal trees. The increase in lipids and waxes makes litter more hydrophobic, which then negatively feeds back to its decomposability. Observations on thick layers of relatively undecomposed litter in north-western European conifer plantations support this hypothesis, which has however not yet been formally tested.

Under N deposition, the decomposition of amino sugars of fungal and bacterial origin was retarded compared to ambient conditions in the study by Griepentrog et al. (2014), but increased in the study by Liao et al. (2020), with an amount similar to the increase in SOC. Griepentrog et al. (2014) suggested that increased availability of mineral N reduced the need for mining N-rich residues from microbial necromass, but the fact that meta-analysis did not show a significant effect of N addition on enzymes associated with organic-N acquisition (Jian et al., 2016) suggests that N-mining is not necessarily sensitive to N deposition. Bacterial residues were selectively preserved in the mineral matrix compared to fungal residues in the study by Griepentrog et al. (2014), while the opposite was noted by Liao et al. (2020), with an increase in fungal residues of +11% and that of bacterial residues of +5%. On the other hand, for a tropical forest Zhang et al. (2016b) noted a reduced contribution of fungal necromass to SOC, but a mechanism was not indicated.

5.4 Nitrogen deposition and soil carbon sequestration

As a consequence of N deposition productivity increases, which results in increased litter fall, and decomposition decreases. Consequently, C accumulates in surface litter and SOC, resulting in C sequestration. Janssens et al. (2010) synthesized SOC changes across 41 temperate- and boreal forests and reported an average increase of 19 g C per g N deposited. Hyvönen et al. (2008) and Maaroufi et al. (2015) reported lower SOC sequestration rates per unit N deposited for Norway spruce (13 and 10 kg C kg^{-1} N, respectively). The latter study reported even lower SOC sequestration rates per unit N added for Scots pine with higher C:N ratios, which is consistent with a beneficial effect of N enrichment on litter decomposition at very low N availabilities. Conversely, at N-saturated temperate forests and in tropical forests where the N cycle is typically more open, a major fraction of the added N is lost from the system, which again leads to lower C sequestration rates per unit N deposited (De Vries et al., 2014; Manzoni et al., 2010; Tonnito et al., 2014).

The importance of reduced C cycling in soils, due to retarded decomposition of litter and SOC, is potentially of comparable importance as enhanced sequestration of C in long-lived tissue in wood. It is not clear how important this N-deposition driven enhancement of C sequestration is for climate mitigation, although most authors agree that it likely makes only a minor contribution (Du and de Vries, 2023). Xu et al. (2021), however, came to a different conclusion. Their analysis, spanning a period of 60 years across 369 terrestrial sites, indicated that N deposition increased stocks of SOC with slightly over 4%. This effect became larger over time, with an average SOC increase of 2% in studies lasting less than 3 years, and almost 10% in studies lasting more than 12 years. This additionally sequestered C was recorded especially in the organic layer (on average 15%), and to a smaller extent in mineral soil layers (4%). The authors suggested that this effect was independent of ecosystem type or climate. Based on these analyses, the authors proposed that an addition rate of 33 kg N ha^{-1} $year^{-1}$ to all terrestrial ecosystems during a period of 20 years has the potential to increase SOC by ~300 Pg, or an amazing 15 Pg $year^{-1}$. The amount of N needed for application over N terrestrial ecosystems was not calculated and the practical importance of these claims is for that reason very contestable.

6. Effects dependent on nitrogen forms and doses

6.1 Differential impacts of ammonium and nitrate

The effect of N addition is also dependent on the source of N. Experimental studies have used both inorganic and organic N sources (urea $(NH_2)_2CO$) has repeatedly been applied as a fertilizer to enhance tree growth], but for this review we have omitted the data that pertain to organic-N fertilisers as urea addition also leads to increases in labile organic C. Inorganic amendments have included ammonium (NH_4^+), nitrate (NO_3^-) or ammonium nitrate. Choice for different N sources in an N addition experiment is partly a result of differential N pollution climates, with in Europe reduced forms of N dominating while in North America oxidized forms of N dominated, although this has recently changed with an increasing dominance toward reduced forms (Du, 2016). While effects of reduced and oxidized forms of N on the N cycle have been extensively studied (Gao et al., 2015), these differential effects of N sources are less well known for the C cycle. Based on data by Ramirez et al. (2010) the negative effect caused by N deposition is independent of the form in which the N is applied.

6.2 Feedbacks and tipping points

N enrichment has been shown to have multiple effects on soil C dynamics. Due to feedback mechanisms N addition modifies the balance between positive and negative feedbacks in soil systems, resulting in potential drastic changes in ecosystem processes. Recently Suz et al. (2021) indicated the possibility for tipping points for ectomycorrhizal fungal communities under N enrichment. Their study suggested tipping points in the case of litter decomposition. Support was provided in a study by Forstner et al. (2019). These authors noted limited effects of N addition in two forests, but the increase in phosphatase activity and eigenvector analysis of enzyme stoichiometry indicated increasing microbial P limitation. The authors speculated that, while the limited responses observed indicate microbial physiological plasticity, this plasticity is not infinite and can reach a tipping point when the forest soil systems reach N saturation. Evidence for microbial plasticity or adaptation was also provided by Allison et al. (2013), who demonstrated that microbial communities from N-enriched plots were associated with higher mass loss in litter bags in N addition plots than communities from ambient plots.

7. Conclusions and outlook

7.1 Conclusions

In this review we have shown that N enrichment typically slows down soil C cycling, which could mitigate the growth rate of atmospheric CO_2 levels in a world that is characterized by both climate change and atmospheric eutrophication. More specifically, our review showed.

(1) Nitrogen deposition exerts negative impacts on the abundance and diversity of saprotrophic microbes (fungi, and bacteria) and changes their species composition toward more opportunistic species. Impacts of N deposition on soil fauna are less straightforward.

(2) Concomitant with the decline in microbial biomass, changes in enzyme activity occur, with hydrolytic-enzyme activity increasing and oxidative-enzyme activity decreasing.

(3) Genomics data confirm that N addition downregulates genes for oxidative enzymes.

(4) Litter decomposition rates and soil respiration are also reduced. However, in the initial stages of litter decomposition an enhancement has repeatedly been observed.

(5) There is still uncertainty whether particulate organic C or mineral-associated organic C is more sensitive to N deposition, and it is possible that the relative effect on both pools is dependent on the mycorrhizal status of the forest.

(6) Most effects of N deposition are stronger in experiments of longer duration or where higher N doses have been added. Often effects are stronger in boreal and temperate forest ecosystems than in subtropical and tropical forests. It is unclear whether this difference is driven by climate, or whether climate is a proxy for differences in the dominant mycorrhizal type, with the ectomycorrhizal symbiosis being dominant in boreal and temperate forests, and the arbuscular mycorrhizal symbiosis in (sub-)tropical forests.

(7) Whereas N deposition slows down the C cycle and hence enhances C sequestration, the amounts sequestered seem to be too small to constitute a major contribution to climate change mitigation.

7.2 Outlook

Despite our general understanding of the negative effects of N deposition on soil C cycling, there are still several issues that indicate our incomplete understanding, and this lack of insight hampers the further development of mechanistic insight. In this section we highlight several issues in need of further research. The issues are not ranked, so no priority order is implied.

(1) Many experimental studies have used pulses of high N additions over a short time period (as in forest fertilization programmes), but N deposition is a slow process with small N amounts entering the forest ecosystem on a daily basis. Ecologists have separated between pulse (high amounts added incidentally) and press (lower amounts added more frequently) experiments. Experimentally, pulse experiments are more easy to execute, but it is not always clear whether both types of experiments elicit the same response. However, laboratory experiments are often more press experiments, as regular addition of small amounts during watering is more easily achievable, but the relevance of laboratory experiments to predict how forests behave needs additional reflection (see below). Future studies should for that reason reflect more explicitly upon the ecological realism of the experiments that are designed (Bebber, 2021).

(2) The usual metric to express effects is by N addition rates (kg N ha^{-1} $year^{-1}$), but this is not necessarily the best way. The data by Van Diepen et al. (2015) show that cumulative effects of N deposition showed a highly significant negative correlation with decreases in foliage and litter Mn mass fractions. It should therefore be imperative that studies both indicate the annual amount of N applied, and also the cumulative dose that the soil has received when the measurements were made. Xing et al. (2022b) assessed both the effect of N dose and time on heterotrophic respiration. They noted that under low N addition (20 kg N ha^{-1} $year^{-1}$) heterotrophic respiration initially increased, whereas at higher additions (50−100 kg N ha^{-1} $year^{-1}$) the negative effect of N addition was already visible in the first year. But heterotrophic respiration declined after 4 years and became negative after 8 years, at the low N dose. These data equally support suggestions that cumulative N levels provide a better assessment than annual N addition rates. Similarly, meta-analyses included the effect of duration of the study, but it is likely that the effect of N deposition on decomposition depends primarily on the degradation stage rather than on time, as the actual decomposition rate varies as a function of temperature.

(3) Meta-analyses aggregate results of many studies and calculate an average response to the factor under consideration. While we have included estimates on average effect sizes, a cautionary note is in order (Koricheva and Gurevitch, 2014). Whereas meta-analysis is a very powerful tool to calculate effect sizes over a large number of studies, aggregation potentially hides contrasting effects. This problem is evident in cases of curvilinear responses or where the average sign of the effect switches with increasing N loading, as with litter decomposition rate where its initial N-limited stage responds positively to N deposition, and later stages that are C-limited respond negatively. The meta-analysis by Gill et al. (2021) provides support for an initial rate-enhancing effect of decomposition after N addition (up to 25% mass loss of confined litter), but a long-term negative effect on decomposition (more than 50% of mass loss). However, even in cases where the relation is linear and the response consistently positive (or negative), meta-analyses are not without problems. For instance, the negative effects of N deposition often increase with the duration of the study, and for that reason the average effect size is to a large extent determined by the balance between short-term and long-term studies. A solution for this problem is a subdivision of the data in discrete categories and including additional tests for significant differences between categories. Many meta-analysis have followed that approach. For practical reasons, because the dataset would otherwise be quite unbalanced, individual factors have been treated as independent. But supposed independent factors could still show underlying correlations. It is plausible that experiments on the impacts of N deposition started earlier in Europe and the US than in China, and as a consequence thereof longer-term experiments are more likely associated with colder climate and with an overrepresentation of ectomycorrhizal forests. Disentangling seemingly, but not necessarily independent variables remains urgent to avoid false causal attribution.

(4) While we cannot exclude the possibility that there exist qualitative differences in ecosystem responses to low or high N doses, we think there currently is no evidence for long-term qualitatively different responses in a dose-dependent way. However, in cases of microbial adaptation of plasticity the negative effects of N on C cycling could be largely attenuated (Allison et al., 2013) and such adaptations are more prevalent with lower N additions. Models that include plasticity for CUE and NUE (Wutzler et al., 2017) should therefore be tested for the specific predictions under N enrichment in forests.

(5) An additional problem is that in many studies either effects of N addition on litter properties or on soil properties, but not on both have been reported. This lack of data limits the ability of meta-analytical approaches to disentangle these factors. It is therefore strongly recommended that studies on the effects of N deposition provide data on both changes in soil properties and changes in litter properties (Vicca et al., 2018).

(6) Our current models cannot well predict how reversible effects of N deposition on C cycling are if N enrichment is abated. It is plausible that soil systems exhibit stable states and that therefore a much larger reduction in N deposition is needed for recovery of the soil system. It is possible that even under abatement of N deposition, secondary losses, e.g., of Mn, will hamper recovery. It is therefore necessary to better understand the mechanism underlying reduced Mn availability with higher cumulative N loading. Slow recovery has been demonstrated by Theuerl et al. (2010), who showed that after reduction of N deposition with 65% for a period of 14 years, no effects on oxidative enzymes were registered, a lack of response that the authors attributed to N saturation of their forest.

(7) Current models that describe effects of N addition on C cycling focus on several microbial parameters that play a major role. Models of shifts in microbial-functional group composition, enzymes and enzyme ratios (Chen et al., 2018c, 2019), and changes in CUE (Wieder et al., 2015) can predict the rate-retarding effect of N deposition on C cycles. But linkages between these classes of parameters are underdeveloped. Increases in CUE under the influence of N addition pertains to the first stages of decomposition, where the C:N ratio of the substrate is well above the critical C:N ratio (Manzoni et al., 2010). In such cases higher CUE results in more microbial biomass and subsequently necromass, which ultimately gets stabilized as recalcitrant or mineral-bound SOC of microbial origin. However, with lower litter C:N ratios and concomitant shifts in enzyme ratios, a higher CUE results in a faster decrease of substrate quality, and this subsequently either reduces CUE and/or microbial biomass, but these linkages are apparently not included in current models.

(8) Assessment of effects of N deposition on ecosystem C pools need to further investigate the relationships between enhanced aboveground and belowground C storage. Terrer et al. (2021) indicated a trade-off between aboveground and belowground C storage under elevated CO_2, with ectomycorrhizal ecosystems showing a strong aboveground response together with belowground C losses, whereas arbuscular mycorrhizal ecosystems showed a weak aboveground response together with enhanced C storage belowground. Mineral-associated C increased in arbuscular mycorrhizal ecosystems, but declined in ectomycorrhizal ecosystems, suggesting a mechanism through which ectomycorrhizal fungi enzymatically mine SOC for N, which subsequently drives plant performance, whereas arbuscular mycorrhizal fungi lack that capability. Because plant and fungal responses to N deposition differ between ectomycorrhizal and arbuscular mycorrhizal ecosystems, it is equally important to assess relationships between aboveground and belowground C storage under N enrichment.

(9) The negative impact of N deposition on soil C cycling has been suggested to act as a mechanism that potentially mitigates effects of elevated CO_2. By reducing decomposition more soil C is sequestered and this provides a negative feedback to atmospheric CO_2 similar to mechanisms through which elevated CO_2 could increase primary production that results in enhanced C sequestration. However, elevated CO_2 also increases mean annual temperature and this then accelerates SOC dynamics, negating the effect of enhanced C sequestration. Several authors have therefore investigated the temperature sensitivity of decomposition under elevated N levels. Ramirez et al. (2012), Wei et al. (2017), and Zhong et al. (2016) reported decreased temperature sensitivity after N enrichment, both in surface and subsurface soils, and suggested that this lower temperature sensitivity weakens the positive climate feedback. Coucheney et al. (2013) on the other hand reported increased temperature sensitivity after addition, implying a reinforcement of the climate feedback. Stone et al. (2012) finally reported no influence of N on the temperature sensitivity of hydrolytic enzymes and chitinase. Apparently, generalizations are not yet possible. One major issue in assessing temperature sensitivity of decomposition is that studies often compared differences in decomposition rates at two temperatures expressed as ratios. Such experimental assessments then are made over short time periods, as longer-term studies are sensitive to changes in substrate quality during decomposition and changes in substrate availability. The standard method of assessing temperature sensitivity does therefore not allow generalisations and a different approach, where the time needed to decompose a certain fraction at both temperatures is assessed, is required (Conant et al., 2008). However, the studies referred to above did not use this methodology. Assessing temperature sensitivity of decomposition under N enrichment in an unbiased way is therefore a major future research topic. Next to temperature effects, interactions between N enrichment and increased drought merit attention.

(10) In order to increase mechanistic insight, several studies have been based on lab incubations, thereby restricting the study to saprotrophic organisms only. In many forests, however, ectomycorrhizal fungi provide a major part of fungal biomass. Several of these species produce peroxidases that are involved in the oxidation of old SOC. Species with a high oxidative capacity, like species of *Cortinarius*, are generally nitrophobic, therefore changes in SOC after N addition could also be attributable to shifts in the ectomycorrhizal fungal community. Reconciling field studies and lab studies therefore remains imperative.

(11) More generally, differences between forest soils dominated by ectomycorrhizal and arbuscular mycorrhizal trees have hardly been considered as a major factor that explains differential responses of N addition to SOC dynamics. However, the theoretical framework proposed by Phillips et al. (2013) suggests this difference is likely a major factor, and data by Midgley and Phillips (2016) showed substantial differences due to N addition, with a much larger sensitivity for ectomycorrhizal than arbuscular mycorrhizal forests. The contribution by ectomycorrhizal fungi to the oxidative-enzyme production is likely a major factor that explains the differential behavior. The differential disconnect between lignin and cellulose degradation in a mainly arbuscular mycorrhizal and an ectomycorrhizal forest under the influence of N addition, as observed by Grandy et al. (2008) supports a likely role for ectomycorrhizal fungal oxidative-enzyme activity. Carrara et al. (2021) noted significant differences between both types mycorrhizal forests, with N addition causing changes in fungal communities in ectomycorrhizal forest and changes in bacterial communities in arbuscular mycorrhizal forest.

(12) Data show that the amount of soil C sequestered per unit N decreases with higher N loadings (but see Xu et al. (2021) for an alternative interpretation). It remains unknown whether this amount decreases to zero or becomes even negative, that results in C losses, when ecosystems are N saturated.

References

Ågren, G.I., Bosatta, E., Magill, A.H., 2001. Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128, 94–98.

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J.B.H., 2013. Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94, 714–725.

Argiroff, W.A., Zak, D.R., Upchurch, R.A., Salley, S.O., Grandy, A.S., 2019. Anthropogenic N deposition alters soil organic matter biochemistry and microbial communities on decaying fine roots. Global Change Biol. 25, 4369–4382.

Argiroff, W.A., Zak, D.R., Pellitier, P.T., Upchurch, R.A., Belke, J.P., 2022. Decay by ectomycorrhizal fungi couples soil organic matter to nitrogen availability. Ecol. Lett. 25, 391–404.

Averill, C., Waring, B., 2018. Nitrogen limitation of decomposition and decay: how can it occur? Global Change Biol. 24, 1417–1427.

Averill, C., Dietze, M.C., Bhatnagar, J.M., 2018. Continental-scale nitrogen pollution is shifting forest mycorrhizal associations and soil carbon stocks. Global Change Biol. 24, 4544–4553.

Basile-Doelsch, I., Balesdent, J., Pellerin, S., 2020. Reviews and syntheses: the mechanisms underlying carbon storage in soil. Biogeosciences 17, 5223−5242.

Bebber, D.P., Watkinson, S.C., Boddy, L., Darrah, P.R., 2011. Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. Oecologia 167, 1177−1184.

Bebber, D.P., 2021. The gap between atmospheric nitrogen deposition experiments and reality. Sci. Total Environ. 801, 149774.

Bejarano-Castillo, M., Campo, J., Roa-Fuentes, L.L., 2015. Effects of increased nitrogen availability on C and N cycles in tropical forests: a meta-analysis. PLoS One 10, e0144253.

Berg, B., 2000. Initial rates and limit values for decomposition of Scots pine and Norway spruce needle litter: a synthesis for N-fertilized forest stands. Can. J. For. Res. 30, 122−135.

Bonner, M.T.L., Castro, D., Schneider, A.N., Sundström, G., Hurry, V., Street, N.R., Näsholm, T., 2019. Why does nitrogen addition to forest soils inhibit decomposition? Soil Biol. Biochem. 137, 107570.

Bowden, R.D., Davidson, E., Savage, K., Arabia, C., Steudler, P., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. For. Ecol. Manag. 196, 43−56.

Bowden, R.D., Wurzbacher, S.J., Washko, S.E., Wind, L., Rice, A.M., Coble, A.E., Baldauf, N., Johnson, B., Wang, J.J., Simpson, M., Lajtha, K., 2019. Long-term nitrogen addition decreases organic matter decomposition and increases forest soil carbon. Soil Sci. Soc. Am. J. 83, S82−S95.

Burton, A.J., Pregitzer, K.S., Crawford, J.N., Zogg, G.P., Zak, D.R., 2004. Simulated chronic NO$_3^-$ deposition reduces soil respiration in northern hardwood forests. Global Change Biol. 10, 1080−1091.

Carrara, J.E., Walter, C.A., Freedman, Z.B., Hostetler, A.N., Hawkins, J.S., Fernandez, I.F., Brzostek, E.R., 2021. Differences in microbial community response to nitrogen fertilization result in unique enzyme shifts between arbuscular and ectomycorrhizal-dominated soils. Global Change Biol. 27, 2049−2060.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359−2365.

Chen, J., Moorhead, D.L., 2022. Progressively decreased nitrogen-stimulation of soil phosphatase activity with long-term nitrogen addition. Soil Biol. Biochem. 169, 104213.

Chen, J., Sinsabaugh, R.L., 2021. Linking microbial functional gene abundance and soil extracellular enzyme activity: implications for soil carbon dynamics. Global Change Biol. 27, 1322−1325.

Chen, J., Luo, Y.Q., Li, J.W., Zhou, X.H., Cao, J.J., Wang, R.W., Wang, Y.Q., Shelton, S., Jin, Z., Walker, L.M., Feng, Z.Z., Niu, S.L., Feng, W.T., Jian, S.Y., Zhou, L., 2017. Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. Global Change Biol. 23, 1328−1337.

Chen, Z.J., Geng, S.C., Zhang, J.H., Setälä, H., Gu, Y., Wang, F., Zhang, X., Wang, X.X., Han, S.J., 2017. Addition of nitrogen enhances stability of soil organic matter in a temperate forest. Eur. J. Soil Sci. 68, 189−199.

Chen, H., Li, D., Feng, W., Niu, S., Plante, A.F., Luo, Y., Wang, K., 2018a. Different responses of soil organic carbon fractions to additions of nitrogen. Eur. J. Soil Sci. 69, 1098−1104.

Chen, H., Li, D.J., Zhao, J., Zhang, W., Xiao, K.C., Wang, K.L., 2018b. Nitrogen addition aggravates microbial carbon limitation: evidence from ecoenzymatic stoichiometry. Geoderma 329, 61−64.

Chen, J., Luo, Y.Q., Van Groenigen, K.J., Hungate, B.A., Cao, J.J., Zhou, X.H., Wang, R.W., 2018c. A keystone microbial enzyme for nitrogen control of soil carbon storage. Sci. Adv. 4, eaaq1689.

Chen, Y.Z., Chen, J., Luo, Y.Q., 2019. Data-driven ENZYme (DENZY) model represents soil organic carbon dynamics in forests impacted by nitrogen deposition. Soil Biol. Biochem. 138, 107575.

Chen, J., Van Groenigen, K.J., Hungate, B.A., Terrer, C., Van Groenigen, J.W., Maestre, F.T., Ying, S.C., Luo, Y., Jørgensen, U., Sinsabaugh, R.L., Olesen, J.E., Elsgaard, L., 2020. Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems. Global Change Biol. 26, 5077−5086.

Cleveland, C.C., Townsend, A.R., 2006. Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. Proc. Nat. Acad. Sci. USA 103, 10316−10321.

Cleveland, C.C., Townsend, A.R., Taylor, P., Alvarez-Clare, S., Bustamante, M.M.C., Chuyong, G., Dobrowski, S.Z., Grierson, P., Harms, K.E., Houlton, B.Z., Marklein, A., Parton, W., Porder, S., Reed, S.C., Sierra, C.A., Silver, W.L., Tanner, E.V.J., Wieder, W.R., 2011. Relationships among net primary productivity, nutrients and climate in tropical rain forest: a pan-tropical analysis. Ecol. Lett. 14, 939−947.

Conant, R.T., Drijber, R.A., Haddix, M.L., Parton, W.J., Paul, E.A., Plante, A.F., Six, J., Steinweg, J.M., 2008. Sensitivity of organic matter decomposition to warming varies with its quality. Global Change Biol. 14, 868−877.

Cotrufo, M.F., Lavallee, J.M., Zhang, Y., Hansen, P.M., Paustian, K.H., Schipanski, M., Wallenstein, M.D., 2021. In-N-Out: a hierarchical framework to understand and predict soil carbon storage and nitrogen recycling. Global Change Biol. 27, 4465−4468.

Coucheney, E., Strömgren, M., Lerch, T.Z., Herrmann, A.M., 2013. Long-term fertilization of a boreal Norway spruce forest increases the temperature sensitivity of soil organic carbon mineralization. Ecol. Evol. 3, 5177−5188.

Cui, Y.X., Moorhead, D.L., Guo, X.B., Peng, S.S., Wang, Y.Q., Zhang, X.C., Fang, L.C., 2021. Stoichiometric models of microbial metabolic limitation in soil systems. Global Ecol. Biogeogr. 30, 2297−2311.

De Vries, W., Du, E.Z., Butterbach-Bahl, K., 2014. Short and long-term impacts of nitrogen deposition on carbon sequestration by forest ecosystems. Curr. Op. Env. Sust. 9−10, 90−104.

Du, E., de Vries, W., 2023. Impacts of nitrogen deposition on forest productivity and carbon sequestration. In: Du, E., de Vries, W. (Eds.), Atmospheric Nitrogen Deposition in Global Forests: Spatial Variation, Impacts and Management Implications. Academic Press (Chapter 4).

Du, E., 2016. Rise and fall of nitrogen deposition in the United States. Proc. Nat. Acad. Sci. USA 113, E3594−E3595.

Eastman, B.A., Adams, M.B., Peterjohn, P.T., 2022. The path less taken: long-term N additions slow leaf litter decomposition and favor the physical transfer pathway of soil organic matter formation. Soil Biol. Biochem. 166, 108567.

Eisenlord, S.D., Freedman, Z., Zak, D.R., Xue, K., He, Z.L., Zhou, J.Z., 2013. Microbial mechanisms mediating increased soil C storage under elevated atmospheric N deposition. Appl. Environ. Microbiol. 79, 1191−1199.

Entwistle, E.M., Zak, D.R., Edwards, I.P., 2013. Long-term experimental nitrogen deposition alters the composition of the active fungal community in the forest floor. Soil Sci. Soc. Am. J. 77, 1648−1658.

Entwistle, E.M., Zak, D.R., Argiroff, W.A., 2018a. Anthropogenic N deposition increases soil C storage by reducing the relative abundance of lignolytic fungi. Ecol. Monogr. 88, 225−244.

Entwistle, E.M., Romanowicz, K.J., Argiroff, W.A., Freedman, F.B., Morris, J.J., Zak, D.R., 2018b. Anthropogenic N deposition alters the composition of expressed class II fungal peroxidases. Appl. Environ. Microbiol. 84, e02816−e02817.

Fanin, N., Kardol, P., Farrell, M., Nilsson, M.C., Gundale, M.J., Wardle, D.A., 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. Soil Biol. Biochem. 128, 111−114.

Feng, J.G., Zhu, B., 2021. Global patterns and associated drivers of priming effect in response to nutrient addition. Soil Biol. Biochem. 153, 108118.

Fog, K., 1988. The effect of added nitrogen on the rate of decomposition of organic matter. Biol. Rev. 63, 433−462.

Forstner, S.J., Wechselberger, V., Stecher, S., Mueller, S., Keiblinger, K.M., Wanek, W., Schleppi, P., Gundersen, P., Tatzber, M., Gerzabek, M.H., Zechmeister-Boltenstern, S., 2019. Resistant soil microbial communities show signs of increasing phosphorus limitation in two temperate forests after long-term nitrogen addition. Front. For. Glob. Change 2, article 73.

Freedman, Z., Zak, D.R., 2014. Atmospheric N deposition increases bacterial laccase-like multicopper oxidases: implications for organic matter decay. Appl. Environ. Microbiol. 80, 4460−4468.

Freedman, Z.B., Zak, D.R., 2015. Atmospheric N deposition alters connectance, but not functional potential among saprotrophic bacterial communities. Mol. Ecol. 24, 3170−3180.

Freedman, Z.B., Romanowicz, K.J., Upchurch, R.A., Zak, D.R., 2015. Differential responses of total and active soil microbial communities to long-term experimental N deposition. Soil Biol. Biochem. 90, 272−282.

Frey, S.D., Ollinger, S., Nadelhoffer, K., Bowden, R., Brzostek, E., Burton, A., Caldwell, B.A., Crow, S., Goodale, C.L., Grandy, A.S., Finzi, A., Kramer, M.G., Lajtha, K., LeMoine, J., Martin, M., McDowell, W.H., Minocha, R., Sadowsky, J.J., Templer, P.H., Wickings, K., 2014. Chronic nitrogen additions suppress decomposition and sequester soil carbon in temperate forests. Biogeochemistry 121, 305−316.

Fu, X.F., Xu, C.H., Geng, Q.H., Ma, X.C., Zhang, H.G., Cai, B., Hu, G.Q., Xu, X., 2022. Effects of nitrogen application on the decomposition of fine roots in temperate forests: a meta-analysis. Plant Soil 472, 77−89.

Gallo, M.E., Lauber, C.L., Cabaniss, S.E., Waldrop, M.P., Sinsabaugh, R.L., Zak, D.R., 2005. Soil organic matter and litter chemistry response to experimental N deposition in northern temperate deciduous forest ecosystems. Global Change Biol. 11, 1514−1521.

Gan, H.J., Zak, D.R., Hunter, M.D., 2013. Chronic nitrogen deposition alters the structure and function of detrital food webs in a northern hardwood ecosystem. Ecol. Appl. 23, 1311−1321.

Gao, W.L., Yang, H., Kou, L., Li, S.G., 2015. Effects of nitrogen deposition and fertilization on N transformations in forest soils: a review. J. Soils Sediments 15, 863−879.

Gill, A.L., Schilling, J., Hobbie, S.E., 2021. Experimental nitrogen fertilisation globally accelerates, then slows decomposition of leaf litter. Ecol. Lett. 24, 802−811.

Grandy, A.S., Sinsabaugh, R.L., Neff, J.C., Stursova, M., Zak, D.R., 2008. Nitrogen deposition effects on soil organic matter chemistry are linked to variation in enzymes, ecosystems and size fractions. Biogeochemistry 91, 37−49.

Griepentrog, M., Bode, S., Boeckx, P., Hagedorn, F., Heim, A., Schmidt, M.W.I., 2014. Nitrogen deposition promotes the production of new fungal residues but retards the decomposition of old residues in forest soil fractions. Global Change Biol. 20, 327−340.

Halbritter, A.H., De Boeck, H.J., Eycott, A.E., Reinsch, S., Robinson, D.A., Vicca, S., Berauer, B., Christiansen, C.T., Estiarte, M., Grünzweig, J.M., Gya, R., Hansen, K., Jentsch, A., Lee, H., Linder, S., Marshall, J., Peñuelas, J., Kappel Schmidt, I., Stuart-Haëntjens, E., Wilfahrt, P., , the ClimMani Working Group, Vandvik, V., 2020. The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx). Methods Ecol. Evol. 11, 22−37.

Hall, S.J., Huang, W.J., Timokhin, V.I., Hammel, K.E., 2020. Lignin lags, leads, or limits the decomposition of litter and soil organic carbon. Ecology 101, 03113.

Hanson, P., Edwards, N., Garten, C., Andrews, J., 2000. Separating root and soil microbial contributions to soil respiration: a review of methods and observations. Biogeochemistry 48, 115−146.

Hasegawa, S., Marshall, J., Sparrman, T., Näsholm, T., 2021. Decadal nitrogen addition alters chemical composition of soil organic matter in a boreal forest. Geoderma 386, 114906.

Hassett, J.E., Zak, D.R., Blackwood, C.B., Pregitzer, K.S., 2009. Are basidiomycete laccase gene abundance and composition related to reduced lignolytic activity under elevated atmospheric NO_3^- deposition in a northern hardwood forest? Microb. Ecol. 57, 728−739.

Hesse, C.N., Mueller, R.C., Vuyisich, M., Gallegos-Graves, L., Gleasner, C.D., Zak, D.R., Kuskel, C.R., 2015. Forest floor community metatranscriptomes identify fungal and bacterial responses to N deposition in two maple forests. Front. Microbiol. 6 article 337.

Ho, A., Di Lonardo, P., Bodelier, P.L.E., 2017. Revisiting life strategy concepts in environmental microbial ecology. FEMS Microbiol. Ecol. 93, 1−14.

Hobbie, S.E., Eddy, W.C., Buyarski, C.R., Adair, E.C., Ogdahl, M.L., Weisenhorn, P., 2012. Response of decomposing litter and its microbial community to multiple forms of nitrogen enrichment. Ecol. Monogr. 82, 389−405.

Hoffland, E., Kuyper, T.W., Comans, R.N.J., Creamer, R., 2020. Ecofunctionality of organic matter in soils. Plant Soil 455, 1−22.

Hofmockel, K.S., Zak, D.R., Blackwood, C.B., 2007. Does atmospheric NO_3^- deposition alter the abundance and activity of ligninolytic fungi in forest soils? Ecosystems 10, 1278−1286.

Howard, P.J.A., Howard, D.M., 1974. Microbial decomposition of tree and shrub leaf litter. 1. Weight loss and chemical composition of decomposing litter. Oikos 25, 341−352.

Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I., Linder, S., 2008. Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. Biogeochemistry 89, 121−137.

Jackson, R.B., Lajtha, K., Crow, S.E., Hugelius, G., Kramer, M.G., Piñeiro, G., 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. Annu. Rev. Ecol. Evol. Syst. 48, 419−445.

Janssens, I.A., Dieleman, W., Luyssaert, S., Subke, J.-A., Reichstein, M., Ceulemans, R., Ciais, P., Dolman, A.J., Grace, J., Matteucci, G., Papale, D., Pialo, S.L., Schulze, E.-D., Tang, J., Law, B.E., 2010. Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. 3, 315−322.

Jia, X.Y., Zhong, Y.Q.W., Liu, J., Zhu, G.Y., Shangguan, Z.P., Yan, W.M., 2020. Effects of nitrogen enrichment on soil microbial characteristics: from biomass to enzyme activities. Geoderma 336, 114256.

Jian, S.Y., Li, J.W., Chen, J., Wang, G.S., Mayes, M.A., Dzantor, K.E., Hui, D.F., Luo, Y.Q., 2016. Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: a meta-analysis. Soil Biol. Biochem. 101, 32−43.

Jo, I., Fei, S., Oswalt, C.M., Domke, G.M., Phillips, R.P., 2019. Shifts in dominant tree mycorrhizal associations in response to anthropogenic impacts. Sci. Adv. 5, eaav6358.

Kaspari, M., Garcia, M.,N., Harms, K.E., Santana, M., Wright, S.J., Yavitt, J.B., 2008. Multiple nutrients limit litterfall and decomposition in a tropical forest. Ecol. Lett. 11, 35−43.

Keller, A.B., Phillips, R.P., 2019. Leaf litter decay rates differ between mycorrhizal groups in temperate, but not tropical, forests. New Phytol. 222, 556−564.

Keller, A.B., Brzostek, E.R., Craig, M.E., Fisher, J.B., Phillips, R.P., 2021. Root-derived inputs are major contributors to soil carbon in temperate forests, but vary by mycorrhizal type. Ecol. Lett. 24, 626−635.

Klotzbücher, T., Kaiser, K., Guggenberger, G., Katzek, C., Kalbitz, K., 2011. A new conceptual model for the fate of lignin in decomposing plant litter. Ecology 92, 1052−1062.

Knorr, M., Frey, S.D., Curtis, P.S., 2005. Nitrogen additions and litter decomposition: a meta-analysis. Ecology 86, 3252−3257.

Koide, R.T., Fernandez, C.W., Peoples, M.S., 2011. Can ectomycorrhizal colonization of *Pinus resinosa* roots affect their decomposition? New Phytol. 191, 508−514.

Koricheva, J., Gurevitch, J., 2014. Usus and misuses of meta-analysis in plant ecology. J. Ecol. 102, 828−844.

Kou, L., Chen, W.W., Zhang, X.Y., Gao, W.L., Yang, H., Li, D.D., Li, S.G., 2015. Differential responses of needle and branch order-based root decay to nitrogen addition: dominant effects of acid-unhydrolyzable residue and microbial enzymes. Plant Soil 394, 315−327.

Kritzler, U.H., Johnson, D., 2010. Mineralisation of carbon and plant uptake of phosphorus from microbially-derived organic matter in response to 19 years simulated nitrogen deposition. Plant Soil 326, 311−319.

Kuyper, T.W., 2013. Die Auswirkungen von Stickstoffeinträgen auf Artengemeinschaften von Pilzen. Z. Mykol. 79, 565−581.

Kuyper, T.W., 2017. Carbon and energy sources of mycorrhizal fungi: obligate symbionts or latent saprotrophs? In: Johnson, N.C., Gehring, G., Jansa, J. (Eds.), Mycorrhizal Mediation of Soil—Fertility, Structure, and Carbon Storage. Elsevier, Amsterdam, pp. 357−374.

Langley, J.A., Chapman, S.K., Hungate, B.A., 2006. Ectomycorrhizal colonization slows root decomposition: the post-mortem fungal legacy. Ecol. Lett. 9, 955−959.

Lauber, C.L., Sinsabaugh, R.L., Zak, D.R., 2009. Laccase gene composition and relative abundance in oak forest soil is not affected by short-term nitrogen fertilization. Microb. Ecol. 57, 50−57.

LeBauer, D.S., Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. Ecology 89, 371−379.

Li, W.B., Jin, C.J., Guan, D.X., Wang, Q.K., Wang, A.Z., Yuan, F.H., Wu, J.B., 2015. The effects of simulated nitrogen deposition on plant root traits: a meta-analysis. Soil Biol. Biochem. 82, 112−118.

Li, H., Santos, F., Butler, K., Herndon, E., 2021. A critical review on the multiple roles of manganese in stabilizing and destabilizing soil organic matter. Environ. Sci. Technol. 55, 12136−12152.

Liao, S., Tan, S.Y., Peng, Y., Wang, D.Y., Ni, X.Y., Yue, K., Wu, F.Z., Yang, Y.S., 2020. Increased microbial sequestration of soil organic carbon under nitrogen deposition over China's terrestrial ecosystems. Ecol. Proc. 9, 52.

Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I., Hobbie, E.A., 2019. Atmospheric nitrogen deposition impacts on the structure and function of forest mycorrhizal communities: a review. Environ. Pol. 246, 148−162.

Lilleskov, E.A., Kuyper, T.W., Bidartondo, M.I., Hobbie, E.A., 2023. Impacts of nitrogen deposition on forest mycorrhizal communities. In: Du, E., de Vries, W. (Eds.), Atmospheric Nitrogen Deposition in Global Forests: Spatial Variation, Impacts and Management Implications. Academic Press (Chapter 6).

Lindahl, B.D., Tunlid, A., 2015. Ectomycorrhizal fungi—potential organic matter decomposers, yet not saprotrophs. New Phytol. 205, 1443−1447.

Lindahl, B.D., Kyaschenko, J., Varenius, C., Clemmensen, K.E., Dahlberg, A., Karltun, E., Stendahl, J., 2021. A group of ectomycorrhizal fungi restricts organic matter accumulation in boreal forest. Ecol. Lett. 24, 1341−1351.

Liu, J., Wu, N.N., Wang, H., Sun, J.F., Peng, B., Jiang, P., Bai, E., 2016. Nitrogen addition affects chemical compositions of plant tissues, litter and soil organic matter. Ecology 97, 1796−1806.

Liu, Q., Yin, R., Tan, B., You, C.M., Zhang, L., Zhang, J., Xu, Z.F., Schädler, M., Scheu, S., 2021. Nitrogen addition and plant functional type independently modify soil mesofauna effects on litter decomposition. Soil Biol. Biochem. 160, 108340.

Lu, X.F., Hou, E.Q., Guo, J.Y., Gilliam, F.S., Li, J.L., Tang, S.B., Kuang, Y.W., 2021. Nitrogen storage stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: a meta-analysis. Global Change Biol. 27, 2780−2792.

Lu, X.K., Vitousek, P.M., Mao, Q.G., Gilliam, F.S., Luo, Y.Q., Turner, B.L., Zhou, G.Y., Mo, J.M., 2021. Nitrogen deposition accelerates soil carbon sequestration in tropical forests. Proc. Nat. Acad. Sci. USA 118, e2020790118.

Lucas, R.W., Casper, B.B., 2008. Ectomycorrhizal community and extracellular enzyme activity following simulated atmospheric N deposition. Soil Biol. Biochem. 40, 1662−1669.

Luo, M., Moorhead, D.L., Ochoa-Hueso, R., Mueller, C.W., Ying, S.C., Chen, J., 2022. Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling. Funct. Ecol. 36, 2845−2858.

Ma, X., Zhu, B., Nie, Y., Liu, Y., Kuzyakov, Y., 2021. Root and mycorrhizal strategies for nutrient acquisition in forests under nitrogen deposition: a meta-analysis. Soil Biol. Biochem. 163, 108418.

Maaroufi, N.I., Nordin, A., Hasselquist, N.J., Bach, L.H., Palmqvist, K., Gundale, M.J., 2015. Anthropogenic nitrogen deposition enhances carbon sequestration in boreal soils. Global Change Biol. 21, 3169−3180.

Maaroufi, N.I., Nordin, A., Palmqvist, K., Gundale, M.J., 2016. Chronic nitrogen deposition has a minor effect on the quantity and quality of aboveground litter in a boreal forest. PLoS One 11, e0162086.

Maaroufi, N.I., Nordin, A., Palmqvist, K., Gundale, M.J., 2017. Nitrogen enrichment impacts on boreal litter decomposition are driven by changes in soil microbiota rather than litter quality. Sci. Rep. 7, 4083.

Manzoni, S., Trofymow, J.A., Jackson, R.B., Porporato, A., 2010. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecol. Monogr. 80, 89−106.

Mao, J.H., Mao, Q.G., Zheng, M.H., Mo, J.M., 2020. Responses of foliar nutrient status and stoichiometry to nitrogen addition in different ecosystems: a meta-analysis. J. Geophys. Res. Biogeosciences 125, JG005347.

Margida, M.G., Lashermes, G., Moorhead, D.L., 2020. Estimating relative cellulolytic and ligninolytic enzymes activities as functions of lignin and cellulose content in decomposing plant litter. Soil Biol. Biochem. 141, 107689.

Marklein, A.R., Houlton, B.Z., 2012. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol. 193, 696−704.

Midgley, M.G., Phillips, R.P., 2016. Resource stoichiometry and the biogeochemical consequences of nitrogen deposition in a mixed deciduous forest. Ecology 97, 3369−3378.

Moore, J.A.M., Anthony, M.A., Pec, G.J., Trocha, L.K., Trzebny, A., Geyer, K.M., Van Diepen, L.T.A., Frey, S.D., 2021. Fungal community structure and function shifts with atmospheric nitrogen deposition. Global Change Biol. 27, 1349−1364.

Moorhead, D.L., Sinsabaugh, R.L., 2006. A theoretical model of litter decay and microbial interaction. Ecol. Monogr. 76, 151−174.

Moorhead, D.L., Lashermes, G., Sinsabaugh, R.L., 2012. A theoretical model of C- and N-acquiring exoenzyme activities, which balances microbial demands during decomposition. Soil Biol. Biochem. 5, 133−141.

Moorhead, D.L., Lashermes, G., Sinsabaugh, R.L., Weintraub, M.N., 2013a. Calculating co-metabolic costs of lignin decay and their impacts on carbon use efficiency. Soil Biol. Biochem. 66, 17−19.

Moorhead, D.L., Rinkes, Z.L., Sinsabaugh, R.L., Weintraub, M.N., 2013b. Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. Front. Microbiol. 4 article 223.

Moorhead, D.L., Sinsabaugh, R.L., Hill, B.H., Weintraub, M.N., 2016. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. Soil Biol. Biochem. 93, 1−7.

Morrison, E.W., Frey, S.D., Sadowsky, J.J., Van Diepen, L.T.A., Thomas, W.K., Pringle, A., 2016. Chronic nitrogen additions fundamentally restructure the soil fungal community in a temperate forest. Fung. Ecol. 23, 48−57.

Morrison, E.W., Pringle, A., Van Diepen, L.T.A., Frey, S.D., 2018. Simulated nitrogen deposition favors stress-tolerant fungi with low potential for decomposition. Soil Biol. Biochem. 125, 75−85.

Olsson, P., Linder, S., Giesler, R., Högberg, P., 2005. Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Global Change Biol. 10, 1745−1753.

Osono, T., Takeda, H., 2001. Effects of organic chemical quality and mineral nitrogen addition on lignin and holocellulose decomposition of beech leaf litter by *Xylaria* sp. Eur. J. Soil Biol. 37, 17−23.

Phillips, R.P., Brzostek, E., Midgley, M.G., 2013. The mycorrhizal-associated nutrient economy: a new framework for predicting carbon-nutrient couplings in temperate forests. New Phytol. 199, 41−51.

Phillips, R.P., 2007. Fertilization effects on fine-root biomass, rhizosphere microbes and respiratory fluxes in hardwood forest soil. New Phytol. 176, 655−664.

Ramirez, K.S., Craine, J.M., Fierer, N., 2010. Nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied. Soil Biol. Biochem. 42, 2336−2338.

Ramirez, K.S., Craine, J.M., Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. Global Change Biol. 18, 1918−1927.

Rappe-George, M.O., Choma, M., Čapek, P., Börjesson, G., Kastovská, E., Šantrů·ková, H., Gärdenäs, A.I., 2017. Indications that long-term nitrogen loading limits carbon resources for soil microbes. Soil Biol. Biochem. 115, 310−321.

Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269, 341−356.

Read, D.J., 1991. Mycorrhizas in ecosystems. Experientia 47, 376−391.

Riggs, C.E., Hobbie, S.E., 2016. Mechanisms driving the soil organic matter decomposition response to nitrogen enrichment in grassland soils. Soil Biol. Biochem. 99, 54−65.

Rocci, K.S., Lavallee, J.M., Stewart, C.E., Cotrufo, M.F., 2021. Soil organic carbon response to global environmental change depends on its distribution between mineral-associated and particulate organic matter: a meta-analysis. Sci. Total Environ. 793, 148569.

Ryan, M.G., Law, B.E., 2005. Interpreting, measuring, and modeling soil respiration. Biogeochemistry 73, 3−27.

Sardans, J., Alonso, R., Janssens, I.A., Carnicer, J., Veresoglou, S., Rillig, M.C., Fernández-Martínez, M., Sanders, T.G.M., Peñuelas, J., 2016. Foliar and soil concentrations and stoichiometry of nitrogen and phosphorus across European *Pinus sylvestris* forests: relationships with climate, N deposition and tree growth. Funct. Ecol. 30, 676−689.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial-stress response physiology and its implications for ecosystem function. Ecology 88, 1386−1394.

Schwede, D.B., Simpson, D., Dentener, F., Du, E., De Vries, W., 2023. Modelling nitrogen deposition in global forests. In: Du, E., De Vries, W. (Eds.), Atmospheric Nitrogen Deposition in Global Forests: Spatial Variation, Impacts and Management Implications. Academic Press (Chapter 3).

Shaw, E.A., Boot, C.M., Moore, J.C., Wall, D.H., Barron, J.S., 2018. Long-term nitrogen addition shifts the soil nematode community to bacterivore-dominated and reduces its ecological maturity in a subalpine forest. Soil Biol. Biochem. 130, 177−184.

Simmel, J., Bässler, C., Poschlod, P., 2017. Ellenberg indicator values for macromycetes − a methodological approach and first indications. Fung. Ecol. 27, 202−212.

Sinsabaugh, R.L., Shah, J.J.F., 2011. Ecoenzymatic stoichiometry of recalcitrant organic matter decomposition: the growth rate hypothesis in reverse. Biogeochemistry 102, 31−43.

Sinsabaugh, R.L., Carreiro, M.M., Repert, D.A., 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60, 1−24.

Sinsabaugh, R.L., Gallo, M.E., Lauber, C., Waldrop, M.P., Zak, D.R., 2005. Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. Biogeochemistry 75, 201−215.

Sinsabaugh, R.L., Hill, B.H., Shah, J.J.F., 2009. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. Nature 462, 795−798.

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol. Biochem. 42, 391−404.

Spohn, M., 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. Biogeosciences 12, 817−823.

Stendahl, J., Berg, B., Lindahl, B.D., 2017. Manganese availability is negatively associated with carbon storage in northern coniferous forest humus layers. Sci. Rep. 7, 15487.

Stone, M.M., Weiss, M.S., Goodale, C.L., Adams, M.B., Fernandez, I.J., German, D.P., Allison, S.D., 2012. Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Global Change Biol. 18, 1173−1184.

Su, Y., Ma, X.F., Gong, Y.M., Li, K.H., Han, W.X., Liu, X.J., 2021. Contrasting effects of nitrogen addition on litter decomposition in forests and grasslands in China. J. Arid Land 13, 717−729.

Su, Y., Ma, X., Gong, Y., Ahmed, Z., Han, W., Li, K., Liu, X., 2022. Global patterns and drivers of litter decomposition under nitrogen enrichment: a meta-analysis. Front. For. Glob. Change 5, 895774.

Sun, Y., Wang, C.T., Chen, H.Y.H., Ruan, H.H., 2020. Responses of C:N stoichiometry in plants, soil, and microorganisms to nitrogen addition. Plant Soil 456, 277−287.

Suz, L.M., Bidartondo, M.I., Van der Linde, S., Kuyper, T.W., 2021. Ectomycorrhizas and tipping points in forest ecosystems. New Phytol. 231, 1700−1707.

Terrer, C., Phillips, R.P., Hungate, B.A., Rosende, J., Pett-Ridge, J., Craig, M.E., Van Groenigen, K.J., Keenan, T.F., Sulman, B.N., Stocker, B.D., Reich, P.B., Pellegrini, A.F.A., Pendall, E., Zhang, H., Evans, R.D., Carrillo, Y., Fisher, J.B., Van Sundert, K., Vicca, S., Jackson, R.B., 2021. A trade-off between plant and soil carbon storage under elevated CO_2. Nature 591, 599−603.

Theuerl, S., Dörr, N., Guggenberger, G., Langer, U., Kaiser, K., Lamersdorf, N., Buscot, F., 2010. Response of recalcitrant soil substances to reduced N deposition in a spruce forest soil: integrating laccase-encoding genes and lignin decomposition. FEMS Microbiol. Ecol. 73, 166−177.

Thomas, D.C., Zak, D.R., Filley, T.R., 2012. Chronic N deposition does not apparently alter the biochemical composition of forest floor and soil organic matter. Soil Biol. Biochem. 54, 7−13.

Thomas, R.Q., Bonan, G.B., Goodale, C.L., 2013. Insights into mechanisms governing forest carbon response to nitrogen deposition: a model-data comparison using observed responses to nitrogen addition. Biogeosciences 10, 3869−3887.

Tian, D.S., Niu, S.L., 2015. A global analysis of soil acidification caused by nitrogen addition. Environ. Res. Lett. 10, 024019.

Tonitto, C., Goodale, C.L., Weiss, M.S., Frey, S.D., Ollinger, S.V., 2014. The effect of nitrogen addition on soil organic matter dynamics: a model analysis of the Harvard Forest Chronic Nitrogen Amendment Study and soil carbon response to anthropogenic N deposition. Biogeochemistry 117, 431−454.

Treseder, K.K., Alster, C.J., Cat, L.A., Gorris, M.E., Kuhn, A.L., Lovero, K.G., Hagedorn, F., Kerekes, J.F., McHugh, T.A., Solly, E.F., 2021. Nutrient and stress tolerance traits linked to fungal responses to global change: four case studies. Elementa-Sci. Anthrop. 9, 00144.

Treseder, K.K., 2008. Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol. Lett. 11, 1111−1120.

Turlapati, S.A., Minocha, R., Bhiravarasa, P.S., Tisa, L.S., Thomas, W.K., Minocha, S.C., 2013. Chronic N-amended soils exhibit an altered bacterial community structure in Harvard Forest, MA, USA. FEMS Microbiol. Ecol. 83, 478−493.

Vallack, H.W., Leronni, V., Metcalfe, D.B., Högberg, P., Ineson, P., Subke, J.-A., 2012. Application of nitrogen fertilizer to a boreal pine forest has a negative impact on the respiration of ectomycorrhizal hyphae. Plant Soil 352, 405−417.

Van Diepen, L.T.A., Frey, S.D., Sthultz, C.M., Morrison, E.W., Minocha, R., Pringle, A., 2015. Changes in litter quality caused by simulated nitrogen deposition reinforce the N-induced suppression of litter decay. Ecosphere 6, article 205.

Vicca, S., Stocker, B.D., Reed, S., Wieder, W.R., Bahn, M., Fay, P.A., Janssens, I.A., Lambers, H., Peñuelas, J., Piao, S., Rebel, K.T., Sardans, J., Sigurdsson, B.D., Van Sundert, K., Wang, Y.P.P., Zaehle, S., Ciais, P., 2018. Using research networks to create the comprehensive datasets needed to assess nutrient availability as a key determinant of terrestrial carbon cycling. Environ. Res. Lett. 13, 125006.

Vitousek, P.M., 1984. Litterfall, nutrient cycling, and nutrient limitation in tropical forests. Ecology 65, 285−298.

Vivanco, L., Austin, A.T., 2011. Nitrogen addition stimulates forest litter decomposition and disrupts species interactions in Patagonia, Argentina. Global Change Biol. 19, 1963−1974.

Waldrop, M.P., Zak, D.R., 2006. Response of oxidative enzyme activities to nitrogen deposition affects soil concentrations of dissolved organic carbon. Ecosystems 9, 921−933.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecol. Appl. 14, 1172−1177.

Wang, C., Liu, D.W., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biol. Biochem. 120, 126−133.

Wei, H., Chen, X.M., He, J.H., Zhang, J.E., Shen, W.J., 2017. Exogenous nitrogen addition reduced the temperature sensitivity of microbial respiration without altering the microbial community composition. Front. Microbiol. 8 article 2382.

Whalen, E.D., Smith, R.G., Grandy, A.S., Frey, S.D., 2018. Manganese limitation as a mechanism for reduced decomposition in soils under atmospheric nitrogen deposition. Soil Biol. Biochem. 127, 252−263.

Whittinghill, K.A., Currie, W.S., Zak, D.R., Burton, A.J., Pregitzer, K.S., 2012. Anthropogenic N deposition increases soil C Storage by decreasing the extent of litter decay: analysis of field observations with an ecosystem model. Ecosystems 15, 450−461.

Wieder, W.R., Grandy, A.S., Kallenbach, C.M., Taylor, P.G., Bonan, G.B., 2015. Representing life in the Earth system with soil microbial functional traits in the MIMICS model. Geosci. Model Dev. 8, 1789−1808.

Wutzler, T., Zaehle, S., Schrumpf, M., Ahrens, B., Reichstein, M., 2017. Adaptation of microbial resource allocation affects modelled long term soil organic matter and nutrient cycling. Soil Biol. Biochem. 115, 322−336.

Xia, M., Talhelm, A.F., Pregitzer, K.S., 2015. Fine roots are the dominant source of recalcitrant plant litter in sugar maple-dominated northern hardwood forests. New Phytol. 208, 715−726.

Xia, M., Talhelm, A.F., Pregitzer, K.S., 2017. Chronic nitrogen deposition influences the chemical dynamics of leaf litter and fine roots during decomposition. Soil Biol. Biochem. 112, 24−34.

Xia, M., Talhelm, A.F., Pregitzer, K.S., 2018. Long-term simulated atmospheric nitrogen deposition alters leaf and fine root decomposition. Ecosystems 21, 1−14.

Xing, A.J., Du, E.Z., Shen, H.H., Xu, L.C., de Vries, W., Zhao, M.Y., Liu, X.Y., Fang, J.Y., 2022a. Nonlinear responses of ecosystem carbon fluxes to nitrogen deposition in an old-growth boreal forest. Ecol. Lett. 25, 77−88.

Xing, A., Du, E., Shen, H., Xu, L., Zhao, M., Liu, X., Fang, J., 2022b. High-level nitrogen additions accelerate soil respiration reduction over time in a boreal forest. Ecol. Lett. 25, 1869−1877.

Xu, C.H., Xu, X., Ju, C.H., Chen, H.Y.H., Wilsey, B.J., Luo, Y.Q., Fan, W., 2021. Long-term, amplified responses of soil organic carbon to nitrogen addition worldwide. Global Change Biol. 21, 1170−1180.

Yang, H.S., Janssen, B.H., 2000. A mono-component model for carbon mineralization with a dynamic rate constant. Eur. J. Soil Sci. 51, 517−529.

Yang, Y., Chen, H., Gao, H., An, S.S., 2020. Response and driving factors of soil microbial diversity related to global nitrogen deposition. Land Degrad. Dev. 31, 190−204.

Zak, D.R., Freedman, Z.B., Upchurch, R.A., Steffens, M., Kögel-Knabner, I., 2017. Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. Global Change Biol. 23, 933−944.

Zak, D.R., Argiroff, W.A., Freedman, Z.B., Upchurch, R.A., Entwistle, E.M., Romanowicz, K.J., 2019. Anthropogenic N deposition, fungal gene expression, and an increasing carbon sink in the Northern Hemisphere. Ecology 100, e02804.

Zechmeister-Boltenstern, S., Keiblinger, K.M., Mooshammer, M., Peñuelas, J., Richter, A., Sardans, J., Wanek, W., 2015. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. Ecol. Monogr. 85, 133−155.

Zhang, W.D., Chao, L., Yang, Q.P., Wang, Q.K., Fang, Y.T., Wang, S.L., 2016a. Litter quality mediated nitrogen effect on plant litter decomposition regardless of soil fauna presence. Ecology 97, 2834−2843.

Zhang, W., Cui, Y.H., Lu, X.K., Bai, E., He, H.B., Xie, H.T., Liang, C., Zhang, X.D., 2016b. High nitrogen deposition decreases the contribution of fungal residues to soil carbon pools in a tropical forest ecosystem. Soil Biol. Biochem. 97, 211−214.

Zhang, T.A., Chen, H.Y.H., Ruan, H.H., 2018a. Global negative effects of nitrogen deposition on soil microbes. ISME J. 12, 1817−1825.

Zhang, T.A., Luo, Y.Q., Chen, H.Y.H., Ruan, H.H., 2018b. Responses of litter decomposition and nutrient release to N addition: a meta-analysis of terrestrial ecosystems. Appl. Soil Ecol. 128, 35−42.

Zhong, Y.Q.W., Yan, W.M., Shangguan, Z.P., 2016. The effects of nitrogen enrichment on soil CO_2 fluxes depending on temperature and soil properties. Global Ecol. Biogeogr. 25, 475−488.

Zhou, L.Y., Zhou, X.H., Zhang, B.C., Lu, M., Luo, Y.Q., Liu, L.L., Li, B., 2014. Different responses of soil respiration and its components to nitrogen addition among biomes: a meta-analysis. Global Change Biol. 20, 2332−2343.

Zhou, L.Y., Zhou, X.H., Shao, J.J., Nie, Y.Y., He, Y.H., Jiang, L.L., Wu, Z.T., Bai, S.H., 2016. Interactive effects of global change factors on soil respiration and its components: a meta-analysis. Global Change Biol. 22, 3157−3169.

Zhou, Z.H., Wang, C.K., Zheng, M.H., Jiang, L.F., Luo, Y.Q., 2017. Patterns and mechanisms of responses by soil microbial communities to nitrogen addition. Soil Biol. Biochem. 115, 441−443.