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# The soil N cycle: new insights and key challenges

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## Abstract

The study of soil N cycling processes has been, is, and will be at the center of attention in soil science research. The importance of N as a nutrient for all biota; the ever increasing rates of its anthropogenic input in terrestrial (agro)ecosystems; its resultant losses to the environment; and the complexity of the biological, physical, and chemical factors that regulate N cycling processes all contribute to the necessity of further understanding, measurement and mitigation of the soil N cycle. Here, we review important insights with respect to the soil N cycle that have been made over the last decade, and present a personal view on the key challenges for future research (Fig. 1). We identified four key questions with respect to N cycling processes:

1. How large is the contribution of non-symbiotic N fixation in natural systems?
2. How important is nitrifier denitrification and what are its main controlling factors?
3. What is the greenhouse gas mitigation potential and microbiological basis for N<sub>2</sub>O consumption?
4. How can we characterize hot-spots and hot-moments of denitrification?

Furthermore, we propose three questions about proximal controls on N cycling processes:

1. How does functional diversity of soil fauna affect N cycling beyond mineralization?
2. What is the functional relationship between root traits and soil N cycling?
3. To what extent do different types of mycorrhizal symbioses (differentially) affect N cycling?

Finally, we identified a key challenge with respect to modelling:

1. How can advanced <sup>15</sup>N / <sup>18</sup>O tracing models help us to better disentangle gross N transformation rates?

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We postulate that addressing these questions would constitute a comprehensive research agenda with respect to the N cycle for the next decade. Such an agenda would help us to meet future challenges on food and energy security, biodiversity conservation and climate stability.

## 1 Introduction

Mankind's relationship with soil nitrogen (N) has been a long and troubled one. For most of agricultural history, farmers have struggled to upkeep soil fertility levels in their fields, relying mostly on biological N fixation (BNF), decomposition of soil organic matter and redistribution of organic materials to provide N to their crops. With the onset of large-scale application of mineral fertilizers after World War II, the main focus in large parts of the world has gradually shifted towards minimizing harmful losses to the environment resulting from the large amounts of N entering the global food production system (Galloway et al., 2013).

The history of research on the soil N cycle reflects this shift. The study of N cycling processes started after Carl Sprengel's discovery (popularized by Justus Von Liebig) of the importance of N as a factor limiting the growth of crop plants in the mid-19th century (Gorham, 1991). More than 150 years of research has demonstrated that this element limits ecosystem productivity over large areas of the globe and is highly sensitive to changes in temperature, precipitation, atmospheric CO<sub>2</sub> and disturbance regime (Galloway et al., 2008). Since the 1960s, following the realization that excess N has negative effects on water, air and ecosystem and human health (Compton et al., 2011; Davidson et al., 2012), the study of the N cycle has intensified, focusing on N loss pathways next to the more traditional study topics such as plant uptake. Most recently, the realization that the response of ecosystems to global environmental change would to a large extent depend on N dynamics (Van Groenigen et al., 2006; Luo et al., 2011) has generated further interest in the soil N cycle. Clearly, our ability to understand, manage and adapt to food security issues and global environmental change is limited by our

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knowledge of soil N cycling processes: their nature, size and dynamics in response to a myriad of environmental factors.

The increased need for information on soil N cycle process rates has coincided with a revolution in the ability to characterize the microbial communities that carry out these processes using molecular techniques. This revolution has been both a help and a hindrance to the effort to quantify process rates. While efforts to extract DNA and RNA and to define microbial communities and diversity have produced fascinating new information on the agents that carry out ever-more complex soil N cycling processes (Isobe and Ohte, 2014), we still lack basic information on the rates of several key processes, and the extent to which they are controlled by biotic interactions in the rhizosphere.

The need for more information on soil N cycling process rates is highlighted by large amounts of “missing N” that dominate N balances at all scales. Inputs of N through fertilization, BNF, atmospheric deposition and human- and animal waste have been found to be substantially higher than hydrological outputs of N in many studies, at many scales (Howarth et al., 1996; Boyer et al., 2002; Groffman, 2008). There is much uncertainty about the fate of this excess N (Van Breemen et al., 2002). Is it stored in soils or vegetation? Is it converted to gas, and if so, in which forms? This uncertainty is particularly compelling in agricultural systems which receive high rates of N input, causing great concern about the air and water quality impacts of these N exports (Davidson et al., 2012). In other areas, there is concern about missing N inputs. Unexplained accumulation of N in aggrading forests (Bernal et al., 2012; Yanai et al., 2013) or in vegetation exposed to elevated levels of atmospheric CO<sub>2</sub> (Zak et al., 2003; Finzi et al., 2007) suggest unmeasured inputs of N via BNF (Cleveland et al., 2010) or uncharacterized mechanisms of soil N turnover and mineralization (Drake et al., 2011; Phillips et al., 2011, 2012).

Here, we review important insights with respect to the soil N cycle that have been made over the last decade, and present our view on the key challenges for future soil research. Although other nutrient cycles can have strong effects on all aspects of the N

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cycle (e.g. Baral et al., 2014), we consider stoichiometric relations to be mostly outside the scope of this paper and do not exhaustively review them.

All authors agree with the contents of the final manuscript; however, freedom has been given to express a somewhat personal view on developments within our respective fields of expertise (see Sect. “Author Contributions”). As such, we hope that our paper will spark discussion and inspire further research on the elusive aspect of soil N cycling.

The eight topics which we address (Fig. 1) encompass basic processes (Sect. 2), proximal controls (Sect. 3) and methodology (Sect. 4). With regard to processes, we first (Sect. 2.1) focus on BNF in natural systems, especially discussing uncertainties with respect to free-living  $N_2$  fixers. Subsequently (Sects. 2.2 and 2.3) we discuss two important but elusive pathways; nitrifier denitrification and  $N_2O$  reduction. We end the section on processes with discussing challenges with respect to measuring denitrification hot-spots and hot-moments (Sect. 2.4). We then focus on proximal controls, starting with the effects that soil fauna can exert on the N cycle through trophic interactions and ecosystem engineering (Sect. 3.1). We then discuss proximal controls by plant roots and litter deposition (Sect. 3.2) as well as by different mycorrhizal symbioses (Sect. 3.3). We end with discussing advanced stable isotope modeling tools to better understand gross N transformations (Sect. 4).

This paper is not meant as a comprehensive literature review of soil N cycling research in the past. Instead, we have tried to be judicious with respect to referencing older studies, only citing some key papers and focusing instead on more recent work with the aim of stimulating debate with respect to the current soil N research agenda.

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## 2 Emerging insights on specific N cycling processes

### 2.1 N<sub>2</sub> fixation

An important share of bioavailable N enters the biosphere via biological fixation of atmospheric N<sub>2</sub> (BNF) (Vitousek et al., 2013). Biological N fixation can be natural (e.g. N<sub>2</sub> fixing trees that are present in forest ecosystems) or anthropogenic (e.g. N<sub>2</sub> fixation by leguminous agricultural crops). Two types of BNF, both using the nitrogenase enzyme, are present in nature: symbiotic N<sub>2</sub> fixation (S-BNF) and free-living N<sub>2</sub> fixation (F-BNF). Symbiotic N<sub>2</sub> fixation is here defined as the infection of plant roots by bacteria – such as *Rhizobia*, *Bradyrhizobia* or actinomycetes – followed by the formation of nodules. All other forms of BNF are regarded as free-living N<sub>2</sub> fixation (including e.g. fixation by bacteria in soil and litter, but also N-fixation in lichens) (Reed et al., 2011).

Nitrogen demand in young successional tropical forest is high. The large fraction of leguminous plant species that forms symbiosis with N<sub>2</sub>-fixing bacteria has recently been identified as a key element of functional diversity to overcome ecosystem-scale N deficiencies in tropical forest successions (Batterman et al., 2013a). Symbiotic fixation is thus considered to relieve N limitations and safeguard forest regrowth and CO<sub>2</sub>-accrual as an ecosystem service. Nevertheless, S-BNF has also been postulated as the reason why mature tropical forest, having a lower N-demand than early succession stands, become relatively rich in N and as a consequence loose (sometimes large amounts of) bioavailable N (Hedin et al., 2009) via NO<sub>3</sub><sup>-</sup> leaching (e.g. Brookshire et al., 2012) or gaseous N loss (e.g. Werner et al., 2007).

However, a plant-level physiological perspective counters this assumption, as numerous experiments have shown that symbiotic S-BNF by leguminous species is mostly facultative and down-regulated when located in an N-rich environment. Tropical leguminous species thus have the potential to fix atmospheric N<sub>2</sub>, but it is likely that they only do so actively in young forest successions or disturbed ecosystems, and far less in mature forests. Secondly, only a part of the *Fabaceae* family have nodule-forming capacities (mainly belonging to the *Mimosoideae* and *Papilionoideae* subfamilies). This

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consideration decreases the omnipresence and abundance of potential N-fixers in tropical forests, making their role as a vital chain in the tropical N-cycle less credible. Therefore, Hedin et al. (2009) have suggested a possible mechanism for explaining this tropical N paradox via a “leaky nitrostat model” (Fig. 2). This concept brings forward the importance of F-BNF, which is hypothesized to take place, even in N-rich ecosystems, in localized N-poor microsites, such as litter layers, topsoil, canopy leaves, lichens or bryophytes on stems, etc. Combined, these free-living  $N_2$  fixers would bring high amounts of N in the system, resulting in high N availability. However, spatially explicit data are virtually absent and largely based on geographically biased, indirect measurements using the acetylene reduction assay rather than direct  $^{15}N_2$  incubation measurements.

A recent spatial sampling method to assess total BNF indicated that tropical forest BNF is likely much lower than previously assumed (Sullivan et al., 2014). These authors reported mean rates of total BNF in primary tropical forests of  $1.2 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ , while previous empirical or modeled data ranged between 11.7 and  $31.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ . Secondary successional forests, as mentioned above, had higher total BNF than primary forest ( $6.2\text{--}14.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ ). Sullivan et al. (2014) proposed a time-integrated total BNF rate of  $5.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$  for primary forest in Costa Rica, of which 20–50% is attributed to S-BNF. It remains to be shown whether this BNF rate from primary tropical forest and proportions between S-BNF and F-BNF are valid for the pan-tropics. But if total BNF in tropical forests is indeed much lower than previously thought, this will fundamentally alter our assessment of tropical forest N cycles and the relative contribution of anthropogenic inputs (Sullivan et al., 2014). There is indeed emerging evidence that anthropogenic N deposition in tropical ecosystems is more substantial than assumed, as a result of biomass burning, dust and biogenic deposition (Chen et al., 2010; European Commission-Joint Research Center, 2014; Cizungu et al., unpublished data). Hence, the relative contribution of human perturbation (e.g. wild fire, livestock fossil fuel combustion) to the tropical N cycle is likely much larger and warrants careful attention, e.g., by increasing N deposition measurement networks in tropical forests





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and Focht, 1985; Schmidt et al., 2004), but always in pure cultures. Despite suggestions that nitrifier denitrification could be an important contributor to soil  $\text{N}_2\text{O}$  emissions (Granli and Bøckman, 1994; Webster and Hopkins, 1996), and that conventional methods of “nitrification  $\text{N}_2\text{O}$ ” measurements such as  $^{15}\text{N}$  tracing or inhibition with  $\text{O}_2$  or acetylene might actually include nitrifier denitrification (Granli and Bøckman, 1994; Mosier et al., 1998), proof of its occurrence in actual soils has remained elusive.

The main challenge to evaluating the importance of nitrifier denitrification in soils is *methodology*. As the N in  $\text{N}_2\text{O}$  produced from both nitrification and nitrifier denitrification originates from the same  $\text{NH}_4^+$  pool, it is impossible to distinguish between these two processes with conventional  $^{15}\text{N}$  tracing methods (Stevens et al., 1997) alone. Methods using inhibition of specific steps of (de)nitrification were proposed as a method to quantify nitrifier denitrification (Webster and Hopkins, 1996), but a series of studies showed that inhibition was unreliable due to problems with effectiveness and selectiveness (Tilsner et al., 2003; Beaumont et al., 2004; Wrage et al., 2004a, b).

Various efforts have been undertaken to employ advanced stable isotope analysis to determine the contribution of nitrifier denitrification as an  $\text{N}_2\text{O}$  source. Sutka et al. (2006) suggested that the intramolecular distribution of  $^{15}\text{N}$  within the asymmetrical  $\text{N}_2\text{O}$  molecule (site preference) might be employed. In monoculture studies, they showed that the site preference signature of nitrifier denitrification and denitrification differed significantly from that of classical nitrification (Sutka et al., 2006) as well as fungal denitrification (Ostrom and Ostrom, 2011). However, in a recent assessment Decock and Six (2013) concluded that huge challenges remain (related to process rates, heterogeneity, unaccounted-for processes, among others) before such an analysis can be reliably applied to soils. They conclude that analysis of site preference will likely remain a qualitative indicator of mechanisms underlying  $\text{N}_2\text{O}$  emissions, and recommend more studies to systematically characterize variation in site preference as a function of ecosystem, soil parameters as well as biogeochemical processes. Such studies are currently being conducted (e.g. Koster et al., 2013; Lewicka-Szczebak et al., 2014; Yano et al., 2014).

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Wrage et al. (2005) proposed an alternative method based on artificially enriched stable isotope tracing. They combined  $^{15}\text{N}$  with  $^{18}\text{O}$  tracing to isolate nitrifier denitrification, utilizing the fact that all O in nitrifier-derived  $\text{N}_2\text{O}$  originates from  $\text{O}_2$ , but half of the O from nitrifier denitrification is derived from  $\text{H}_2\text{O}$ . However, their method, employing  $^{18}\text{O}$ -enriched  $\text{H}_2\text{O}$  as well as  $^{15}\text{N}\text{-NO}_3^-$  and  $^{15}\text{N}\text{-NH}_4^+$ , did not take into account O exchange between  $\text{H}_2\text{O}$  and intermediates of the (de)nitrification pathways (Kool et al., 2007, 2009). This exchange can be quantified using  $^{18}\text{O}$  labelled  $\text{NO}_3^-$  (Kool et al., 2010, 2011b). With the help of a revised method, Kool et al. (2011a) showed that nitrifier denitrification exceeded “classical nitrification” as a dominant source of  $\text{NH}_4^+$ -derived  $\text{N}_2\text{O}$  emission, and was a dominant pathway of total  $\text{N}_2\text{O}$  production at low and intermediate soil moisture contents. Other studies using this method have confirmed that nitrifier denitrification was indeed the dominant pathway for  $\text{NH}_4^+$  derived  $\text{N}_2\text{O}$  emissions (Zhu et al., 2013).

With terminology established and a method developed, nitrifier denitrification is now ready to be studied in detail in soils. However, methodological constraints still exist, as the dual isotope method is elaborate and includes a relatively large number of assumptions. These constraints will have to be addressed in the future.

### 2.3 Nitrous oxide consumption

Net consumption of atmospheric  $\text{N}_2\text{O}$  is enzymatically and energetically feasible. Consumption of  $\text{N}_2\text{O}$  has been sporadically reported for several terrestrial ecosystems, but mostly for wetlands and peatlands. A recent review by Schlesinger (2013) reports a net  $\text{N}_2\text{O}$  uptake range of  $< 1\text{--}207 \mu\text{g N m}^{-2} \text{h}^{-1}$ , but almost all uptake fluxes fall between 1 and  $10 \mu\text{g N m}^{-2} \text{h}^{-1}$ , with a median of  $4 \mu\text{g N m}^{-2} \text{h}^{-1}$ . Another recent review (Majumdar, 2013) reported in situ  $\text{N}_2\text{O}$  consumption rates in rice fields ranging from  $0.13\text{--}191 \mu\text{g N m}^{-2} \text{h}^{-1}$ . Yang et al. (2011) developed an  $^{15}\text{N}_2\text{O}$  isotope dilution method that allows for calculation of gross  $\text{N}_2\text{O}$  production and consumption rates. These authors observed a relative  $\text{N}_2\text{O}$  yield of 0.84, indicating that 16% of the gross  $\text{N}_2\text{O}$

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production was consumed in situ. Hence, both net atmospheric and in situ  $N_2O$  consumption occurs in soil reducing both atmospheric lifetimes and net  $N_2O$  effluxes. However, Well and Butterbach-Bahl (2013) question the validity of the latter experimental approach. The latest IPCC report (Stocker et al., 2013) mentions a global surface  $N_2O$  sink of 0–1 Tg  $N_2O-N yr^{-1}$ . This sink strength is not sufficient to explain the imbalance between global  $N_2O$  sources and sinks (Schlesinger, 2013).

Based on recent evidence from the literature we have identified three possible routes for  $N_2O$  consumption. First, in addition to the “typical” nitrous oxide reductase (nosZ I) that reduces  $N_2O$  during denitrification, a recently identified microbial guild is suggested to mediate the soil  $N_2O$  sink (Sanford et al., 2012; Jones et al., 2014). Newly discovered non-denitrifier, “atypical”  $N_2O$  reductase (nosZ II) gene diversity and abundance potentially play a significant role in  $N_2O$  consumption in soil. Orellana et al. (2014) indicated that “atypical” nosZ outnumber typical nosZ in soil.

Second, some bacteria that perform dissimilatory nitrate reduction to ammonia (DNRA) are capable of  $N_2O$  reduction to  $N_2$  as they carry a *nos* gene encoding for  $N_2O$  reductase ( $N_2OR$ ) (Simon et al., 2004). Mania et al. (2014) indicated that, depending on the environmental conditions, these bacteria may reduce  $N_2O$  that is provided by other bacteria or that they produced themselves as a by-product during DNRA.

Third, there is evidence that both direct assimilatory  $N_2O$  fixation via nitrogenase (Vieten et al., 2008; Ishii et al., 2011; Farías et al., 2013) or indirect  $N_2O$  fixation via a combination of  $N_2O$  reduction and  $N_2$  fixation can account for  $N_2O$  consumption. Itakura et al. (2013) showed that inoculation of soil grown with soybean with a non-genetically modified mutant of *Bradyrhizobium japonicum* with a higher  $N_2O$  reductase activity (nosZ++) reduced  $N_2O$  emission. In farm-scale experiments on an Andosol, an  $N_2O$  mitigation of ca. 55% was achieved with such inoculation. Desloover et al. (2014) identified a *Pseudomonas stutzeri* strain that was able to grow on  $N_2O$  as the only source of N and electron acceptor. *Pseudomonas stutzeri* is known to possess both nitrogenase and nitrous oxide reductase (nosZ I) (Pomowski et al., 2011). A  $^{15}N$  labelling study showed that  $N_2O$  is immobilized into microbial biomass via  $N_2O$  reduction

to N<sub>2</sub> followed by re-uptake of the released N<sub>2</sub> and subsequent fixation into NH<sub>4</sub><sup>+</sup> via nitrogenase (Desloover et al., 2014).

In conclusion, five possible pathways for N<sub>2</sub>O consumption have been identified (Fig. 4): (1) dissimilatory N<sub>2</sub>O reduction to N<sub>2</sub> via typical, denitrifier nosZ I, (2) atypical, non-denitrifier nosZ II, (3) during DNRA, (4) direct assimilatory N<sub>2</sub>O fixation via nitrogenase to NH<sub>3</sub>, and (5) indirect assimilatory N<sub>2</sub>O fixation (N<sub>2</sub>O reduction to N<sub>2</sub> followed by N<sub>2</sub> fixation). Clearly, NO<sub>3</sub><sup>-</sup> reduction in soil is handled by a network of actors (Kraft et al., 2011) and has a more modular character than the classical linear presentation of denitrifying enzymes suggests (Simon and Klotz, 2013). Moreover, a high degree of metabolic versatility is observed for many organisms; genes encoding for denitrification, DNRA, and atmospheric N fixation have, for instance, been found in a single bacterial species (Simon, 2002; Mania et al., 2014). Finally, Verbaendert et al. (2014) showed that molecular tools that have been developed to identify denitrifying bacteria are biased towards gram-positive denitrifiers. Hence, we propose that assessment of novel gene expressions in conjunction with the quantification of N<sub>2</sub>O consumption in various soil types is required to advance our understanding of microbial and physicochemical controls on N<sub>2</sub>O consumption, and ultimately to develop improved biogeochemical models of soil N<sub>2</sub>O sink function.

## 2.4 Denitrification

Perhaps the most poorly understood process in the N cycle is denitrification, the anaerobic microbial conversion of the nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) to the gases nitric oxide (NO), nitrous oxide (N<sub>2</sub>O) and dinitrogen (N<sub>2</sub>) (Seitzinger et al., 2006; Groffman, 2012). This process is of great interest because it can significantly reduce pools of reactive N (and thus productivity) in ecosystems and because NO<sub>3</sub><sup>-</sup>, NO and N<sub>2</sub>O cause diverse air and water pollution problems (Davidson et al., 2012). Denitrification is difficult to quantify because of problematic measurement techniques (especially for its end product N<sub>2</sub>), high spatial and temporal variability, and a lack of methods for scaling point measurements to larger areas (e.g. Groffman et al., 2006). A particular challenge is

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the fact that small areas (hotspots) and brief periods (hot moments) frequently account for a high percentage of N gas flux activity, and that it is increasingly recognized that denitrification is in many ways a modular rather than a singular process. This presents a variety of problems related to measurement, modelling and scaling (Groffman et al., 2009). Global mass balance analyses (Seitzinger et al., 2006) suggest that the biggest global sink for anthropogenic N must be terrestrial denitrification, yet there are few direct measurements to support these results. Modelling efforts estimate that global N<sub>2</sub> production from denitrification may increase from 96 Tg yr<sup>-1</sup> in 2000 to 142 Tg yr<sup>-1</sup> in 2050 due to increased N inputs in the global agricultural system (Bouwman et al., 2013). Questions about “missing N” and denitrification are particularly dramatic and compelling in agricultural ecosystems, landscapes and regions, where most industrially derived N is applied and the opportunity for large terrestrial denitrification fluxes exists.

Addressing the challenge of denitrification requires advances in three main areas; (1) improved methods for quantifying N gas fluxes, (2) experimental designs that incorporate hotspot and hot moment phenomena, and (3) approaches for temporal and spatial scaling that account for hotspot and hot moment phenomena at multiple scales.

Denitrification has always been a challenging process to measure (Groffman et al., 2006), primarily due to the difficulty of quantifying the flux of N<sub>2</sub> from soil against the high natural atmospheric background of this gas (Yang and Silver, 2012; Yang et al., 2014). Most denitrification methods therefore involve alteration of physical or chemical conditions through the use of inhibitors (e.g., acetylene) or amendments (e.g., <sup>15</sup>N) that produce inaccurate or unrealistic estimates of rates. However, there have been recent advances in methods for quantifying N<sub>2</sub> flux and in isotope-based methods that provide area and time-integrated denitrification estimates that are more relevant to ecosystem-scale questions.

Our understanding of the N<sub>2</sub> flux associated with denitrification has been improved by the development of soil core-based gas recirculation systems that involve replacement of the natural soil N<sub>2</sub> / O<sub>2</sub> atmosphere with a He / O<sub>2</sub> atmosphere, followed by direct

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measurement of  $N_2$  and  $N_2O$  production as well as their ratio (Swerts et al., 1995; e.g. Wang et al., 2011; Kulkarni et al., 2014). It is important to note that these new methods are based on extracted soil cores, incubated over extended periods, which can create artificial conditions (Frank and Groffman, 2009). However, some confidence in the flux estimates from cores can be developed by comparing estimates of  $CO_2$  and  $N_2O$  fluxes in the cores and in situ field chambers.

The new soil core incubation systems, along with new soil  $O_2$  sensors, have also advanced our understanding of hot moments of denitrification. Because it is possible to vary the  $O_2$  concentration of the recirculation stream in the new incubation systems, denitrification versus  $O_2$  relationships can be established and linked with continuous estimates of soil  $O_2$  from the new sensors to produce continuous estimates of flux (Burgin and Groffman, 2012; Duncan et al., 2013). Recent studies have shown that these relationships are more complex than previously thought. For example, in northern hardwood forests in north-eastern North America, denitrification rates have been found to be higher at 5 or 10 %  $O_2$  than under completely anaerobic conditions, suggesting that there is tight coupling between  $NO_3^-$  production by nitrification and denitrification in these soils (Morse et al., 2014a).

As our ability to quantify denitrification has improved, our understanding of the factors that control the occurrence of hotspots and hot moments of activity has also increased. Riparian zones have been studied in this regard for several decades (e.g. Lowrance et al., 1997; Mayer et al., 2007). This has resulted in efforts to protect and restore riparian zones to decrease N delivery to receiving waters in many locations. Still, there is great uncertainty about just how much N is denitrified in riparian zones and through other N control practices, and how much N remains in the soils and vegetation of these areas where it is susceptible to later conversion back to  $NO_3^-$  or  $N_2O$  (Woli et al., 2010).

More recently, there has been recognition of the potential for hotspots and hot moments denitrification to occur within crop fields. Periods of transient saturation low in the soil profile can support significant amounts of denitrification that are missed in sampling programs that focus on surface soils (Werner et al., 2011; Morse et al., 2014b).

Areas of wet soil, low soil O<sub>2</sub> and possibly high denitrification are also common at the transition between fall and winter and between winter and spring (Walter et al., 2000).

Estimates of denitrification produced by direct measurement in soil cores can be validated using isotope measurements and models. Shifts in <sup>15</sup>N-NO<sub>3</sub><sup>-</sup> have been used to indicate denitrification in soils, riparian zones, agricultural streams, and large rivers (e.g. Kellman and Hillaire-Marcel, 1998; Vidon and Hill, 2004). Dual natural isotope (δ<sup>18</sup>O- and δ<sup>15</sup>NO<sub>3</sub><sup>-</sup>) analysis has been used to estimate denitrification in aquifers (Wassenaar, 1995), agricultural (Burns et al., 2009) and urban (Kaushal et al., 2011) catchments as well as in tropical forest soils (Houlton et al., 2006).

The time is thus ripe for ecosystem, landscape and regional-scale studies of denitrification. We have new methods capable of producing well constrained estimates of denitrification at the ecosystem scale, new ideas about the occurrence of hotspots and hot moments at ecosystem and landscape scales, and powerful new tools for extrapolation and validation at regional and continental scales.

### 3 Proximal controls of N cycling processes

#### 3.1 Soil fauna

Until recently, the influence of fauna other than humans on the soil N cycle has been mostly neglected. Nitrogen transformation processes and -loss pathways have almost exclusively been related to the interplay between microbial dynamics in the soil and abiotic factors. At first glance this seems logical: micro-organisms dominate the biomass of soil life to a large degree, and many conversions in the N cycle (e.g. nitrification, denitrification, nitrifier-denitrification, N fixation, DNRA) are the exclusive domain of micro-organisms. Biochemical as well as physical processes such as N leaching are controlled by abiotic factors (e.g. pH, porosity and temperature). In turn, both microbial dynamics and abiotic factors can be changed by human influences such as N

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deposition in natural systems and fertilization, liming and soil tillage in agricultural systems (Fig. 5a).

What important role do soil fauna then have in the N cycle? Like the effect of humans, their role can be dramatic but is essentially indirect: through trophic interactions and burrowing activities they may strongly affect microbial dynamics in the soil as well as soil physical properties (Fig. 5b).

The only part of the soil N cycle where the role of soil fauna has been reasonably well established is N mineralization and subsequent plant uptake. Soil fauna affects N mineralization by a combination of activities, including trophic interactions (grazing on micro-organisms, predation) as well as fragmentation of organic matter, mixing organic matter into the soil, excreting nutrient-rich compounds and dispersing microbial propagules (Bardgett and Chan, 1999).

In a literature study across natural and agricultural systems, Verhoef and Brussaard (1990) found a relatively stable faunal contribution to N mineralization of around 30%. Different functional groups of soil fauna, however, contribute to N mineralization differently, with the largest contributions provided by bacterial-feeding microfauna (nematodes and amoeba), followed by earthworms and potworms, and minor contributions by fungal-feeding nematodes and micro-arthropods (De Rooter et al., 1993). Among meso- and macro-fauna, the role of earthworms has been most extensively studied (e.g. Postma-Blaauw et al., 2006; Van Groenigen et al., 2014). As “ecosystem engineers”, they are well-known to affect soil structure and litter redistribution, thereby affecting many aspects of the N cycle as well as other soil processes (Shipitalo and Le Bayon, 2004; Blouin et al., 2013). In a recent meta-analysis, Van Groenigen et al. (2014) showed that in agricultural systems earthworms increase crop yield on average by 25%. This effect was consistent between different functional groups of earthworms, but increased with earthworm density and crop residue application rates. Because this beneficial effect disappeared with adequate N fertilization, it was mainly ascribed to increased N mineralization from crop residue and soil organic matter. In tropical ecosystems soil-feeding termites are known to have a similarly large

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impact on N mineralization (Ji and Brune, 2006). Termites are also able to volatilize ammonia from their gut as well as from their faeces. However, this has only been shown to lead to high  $\text{NH}_3$  concentrations in their nest atmosphere. It is not yet clear whether the  $\text{NH}_3$  accumulating in the internal nest atmosphere can escape into the ambient air (Ji and Brune, 2006).

The effect of faunal diversity rather than single faunal groups is complex. Combinations of functionally dissimilar soil fauna can increase the N-mineralization rate due to facilitative interactions (Heemsbergen et al., 2004). These include one group benefiting from the activity of another group, for example through changes in soil structure or litter shredding by isopods promoting microbial growth (Wardle, 2006). Yet, competitive interactions may also positively influence mineralization rates (Loreau, 1998). For instance, predatory mites in the soil feed on fungivorous mites and potworms as well as springtails and nematodes (De Ruiter et al., 1995), and can thereby influence microbial activities through trophic cascades (induced positive effects on microbes by feeding on microbial feeders). Even though empirical evidence of such trophic cascades in soil food webs is scarce (Mikola and Setälä, 1998; Bardgett and Wardle, 2010), the presence of predatory mites can potentially influence the behavior of fungivorous mites and potworms in terms of their feeding rate and spatial distribution. Such interactions (both facilitative and competitive), within and across trophic levels, have not yet been explored for most N cycling processes, including N loss pathways.

Among the relatively few studies that have focused on processes other than N mineralization, earthworms are again by far the most studied group. They have been shown to affect microbial N immobilization (Brown et al., 1998) as well as nitrification and denitrification (e.g. Parkin and Berry, 1999; Rizhiya et al., 2007). A growing body of literature shows that earthworms can considerably increase  $\text{N}_2\text{O}$  emissions (Lubbers et al., 2013). A recent meta-analysis on the effect of earthworms on soil greenhouse gas emissions reported an average earthworm-induced increase in  $\text{N}_2\text{O}$  emissions of 42 % (Lubbers et al., 2013). This was hypothesized to be the result of effects on the denitrifier community as well as changes in soil structure affecting gas diffusivity and anaerobicity

(Drake and Horn, 2006, 2007; Nebert et al., 2011). Further, molecular, work is needed to determine what the exact effects are of earthworm activity on microbial producers and consumers of N<sub>2</sub>O.

Evidence for involvement of other faunal groups in these processes is scarce. Potworms, phylogenetically related to earthworms and with similar foraging and burrowing habits (albeit at a smaller scale), have been recognized as vectors for microbial colonization (Rantalainen et al., 2004) and may influence both nitrification and denitrification processes (Van Vliet et al., 2004). High soil NO<sub>3</sub> levels in the presence of potworms have been linked to increased nitrification potential (Liiri et al., 2007). Recent work has shown that trophic interactions involving springtails, fungivorous mites and predatory mites can strongly affect N<sub>2</sub>O emissions (Kuiper et al., 2013; Thakur et al., 2014), although the exact pathways remain unclear – both “real” trophic relations as well as altered behavior due to sensing of the presence of predators may play a role.

Changes in soil structure (porosity, aggregation) by faunal activity can affect soil physical processes as well. Burrowing activities of earthworms may create preferential flow pathways that increase leachate volume and consequently the total leaching loss of inorganic N and dissolved organic N (e.g. Dominguez et al., 2004). Interactions between other soil faunal species have received little attention with regard to their effects on soil physical properties. Smaller fauna such as potworms, springtails, mites and nematodes are often assumed to have negligible direct effects on larger-scale soil structure, because they are usually confined to pre-existing voids in litter or soil (Lee and Foster, 1991; Whalen and Sampedro, 2010). However, these small fauna can significantly alter soil microstructure by producing faecal pellets, and potworms can also increase soil porosity and pore continuity by their burrowing activity (Topoliantz et al., 2000; Van Vliet et al., 2004).

Ultimately, the role of soil fauna, as so much else in the soil, is strongly determined by human activity. In agricultural fields, land management such as tillage can disturb the soil food web and shift soil food web composition by differential sensitivities of the

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soil fauna to tillage (Postma-Blaauw et al., 2012). Application of crop residues, manure or fertilizer can change the soil food web size and structure by the supply of easily available C and N in specific locations and at specific times (Fig. 5). Future efforts to model the effects of soil fauna on N dynamics will have to address both the direct effects of fauna as well as the indirect effects of soil management on faunal communities.

### 3.2 Rhizodeposition and plant traits

Soil microbial communities depend almost exclusively on plant derived resources for their energy and nutrient supply. For a long time, it was presumed that plant litter was the most relevant organic matter input for the soil food web, and that plant effects on soil biogeochemistry were mainly mediated via the indirect impacts of plant inputs on relatively inert soil properties. Therefore, most of our initial understanding of soil biogeochemistry was based on experiments with root-free soils.

The impact of spatially and temporarily dynamic processes occurring in the rhizosphere on N cycling has rarely been considered (Frank and Groffman, 2009; Rütting et al., 2011b). Nevertheless, an important share of the energy for microbial metabolism is delivered by belowground plant parts through root exudation, cell sloughing, and root and mycorrhizal fungal turnover (Nguyen, 2003). Healthy growing roots pass a large proportion of the C they receive to the soil as root exudates. This includes a range of materials, but soluble compounds, consisting of organic acids, carbohydrates and amino acids comprise the largest component (Farrar et al., 2003). The total amount and composition of root exudates varies between plant species and genotypes, and is influenced by plant phenology and environmental conditions (Nguyen, 2003). Moreover, fine root turnover, caused by the production, mortality and decay of short-lived C-rich roots, is another key pathway of significant nutrient flux in terrestrial ecosystems that may equal or even exceed that of above-ground litter fall in certain ecosystems (Gill and Jackson, 2000; Yuan and Chen, 2010).

There are several mechanisms through which plant roots can affect rhizosphere N cycling (reviewed in Paterson, 2003; Dijkstra et al., 2013; Cheng et al., 2014). Often,

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rhizodeposition enhances microbial growth and activity and stimulates production of microbial exoenzymes that mine for more complex soil organic N compounds (Paterson, 2003). Nitrogen immobilized by the microbial community may temporarily reduce soil N availability, but immobilized N can become available in the rhizosphere due to microbial turnover and the grazing of rhizosphere microorganisms by soil micro-fauna (see Sect. 3.1). The quality of rhizodeposition is an important determinant for soil microbial communities; any shifts in their composition may affect decomposition processes through the production of distinct sets of extracellular enzymes (Dennis et al., 2010; Kaiser et al., 2010). Nevertheless, under conditions of low N availability, plant N uptake may limit microbial substrate N availability and reduce microbial growth and decomposition activity (Dijkstra et al., 2010; Blagodatskaya et al., 2014). Moreover, the production of specific metabolites that act as signaling molecules could accelerate or retard soil N cycling if they act upon certain functional microbial taxa (De-la-Pena and Vivanco, 2010). Finally, specific N cycling processes, such as denitrification or N fixation could be altered in the rhizosphere due to altered microbial substrate conditions, encompassing C, O<sub>2</sub> and NO<sub>3</sub><sup>-</sup> availabilities (Philippot et al., 2009). Altogether, rhizodeposition mostly causes an increase in microbial activity and soil N decomposition compared to bulk soils. Nevertheless, nutrient availability in the rhizosphere and competitive interactions between plant and microbial communities may shift the magnitude and direction of N cycling processes, especially those processes performed by phylogenetically less diverse microbial functional groups, such as nitrification and denitrification (Philippot et al., 2009; Dijkstra et al., 2013).

Although the quality and quantity of rhizodeposits clearly influence rhizosphere N cycling, a major challenge lies in determining to what extent plant community characteristics explain the observed variations of rhizosphere impacts (Cheng et al., 2014). Considering the great difficulties in assessing rhizodeposition under field conditions (Pausch et al., 2013a), a prospective approach may involve measuring “soft” plant traits that are relatively easy to observe and quantify (Fry et al., 2014). There are several traits that are good candidates due to their putative intimate relationship with

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rhizodeposition. For example, root exudation is linked to the intensity of canopy photosynthetic activity and photo-assimilate supply (Kuzyakov and Cheng, 2001). Fast-growing, acquisitive plants with high specific leaf area and short life span are thus thought to be associated with a larger rhizosphere effect (Wardle et al., 2004). Because root exudation is concentrated at the apices of the roots and at the nodes where lateral roots emerge (Jaeger et al., 1999), root architectural traits determine the expansion of the rhizosphere and exudate fluxes per unit of root biomass. A densely branched root system with high biomass and a rapid turnover thus contributes large quantities of exudates (Van der Krift et al., 2001). The chemistry of rhizodeposits is a key controlling variable of rhizosphere dynamics, as microbial communities may shift their N use efficiency in response to substrate stoichiometry, leading to changes in soil N cycling fluxes (Moorshammer et al., 2014).

Several studies have examined presumed relationships between N cycling parameters and plant traits, especially of aboveground plant organs (e.g. Wedin and Tilman, 1990; Orwin et al., 2010; Garcia-Palacios et al., 2013; Grigulis et al., 2013). Soil N cycling processes appear to be primarily driven by traits of the most abundant species (the biomass ratio hypotheses; Grime, 1998), although complex effects may arise due to interspecies interactions and non-additive species effects (Grigulis et al., 2013; Pausch et al., 2013b). These studies confirm that plant characteristics, including under-investigated root traits, exert a key control over soil microbial communities, and modify the fundamental physiologies that drive soil N cycling. Nevertheless, the lack of clear-cut relationships between specific plant traits and N cycling parameters indicates the necessity for more research on plant communities to establish consistent links between plant traits and N cycling variables. Understanding such relationships will lead to improved upscaling capabilities, and perhaps ultimately the inclusion of rhizosphere effects in biogeochemical models.

### 3.3 Mycorrhizal associations

This section will focus on the extent to which the main types of mycorrhizal symbioses, arbuscular mycorrhiza and ectomycorrhiza, differentially affect the soil N cycle. Early conceptual models linked the replacement of arbuscular mycorrhizal plants by ectomycorrhizal plants to succession (Read, 1991) or to latitudinal and altitudinal gradients from warmer to colder climates (Read and Perez-Moreno, 2003). This was considered to be driven by shifts from P to N limitation and from mainly inorganic to more organic nutrient cycles. However, Dickie et al. (2013) noted a poor fit between these models and actual data on primary succession and suggested that nutrient limitation shifts from N- to P-limitation in retrogressive succession. Although a new model of general applicability has not yet been proposed, the underlying idea of a fundamental difference between arbuscular mycorrhiza-dominated ecosystems with more open, inorganic nutrient cycles and ectomycorrhiza-dominated ecosystems with more closed, organic nutrient cycles has persisted, especially for forests in temperate regions (Phillips et al., 2013; Bradford, 2014). We note that the same distinction was proposed between bacterial- and fungal-dominated agro-ecosystems by De Vries and Bardgett (2012). Their conceptual model is apparently not applicable for the tropics, where both arbuscular mycorrhizal and ectomycorrhizal forests are characterized by an open N cycle (Kuyper, 2012; Tedersoo et al., 2012). This geographical contrast raises the question to what extent the nature of the mycorrhizal symbiosis is causally relevant for differences in forest ecosystem functioning, or whether plant traits other than the mycorrhizal symbiosis cause these differences. Arguments that the mycorrhizal symbiosis is causally relevant for soil N cycling are connected to the claim that ectomycorrhizal fungi, contrary to arbuscular mycorrhizal fungi, possess extensive saprotrophic activity to mine for N (Koide et al., 2008; Talbot et al., 2008), and therefore could access organic sources of N and phosphorus.

Several authors have compared uptake of various amino acids by arbuscular and ectomycorrhizal plants. The ability to depolymerize large N-containing molecules

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(proteins) into smaller fragments that can be taken up (Schimel and Bennett, 2004) and the ability to increase access to these large molecules, which are often bound to phenolics and other recalcitrant compounds, have been mainly studied for ectomycorrhizal fungi. Talbot and Treseder (2010) demonstrated widespread ability among ectomycorrhizal fungi to take up amino acids and noted that the relative benefit of the symbiosis was largest for the most common amino acids. Arbuscular mycorrhizal fungi also have widespread ability to take up amino acids (Whiteside et al., 2012), however, the arbuscular mycorrhizal benefit is largest with the least common amino acids. The authors hypothesized that these contrasting patterns of amino acid use may reduce competition for rare amino acids. However, the extent to which this form of niche differentiation would reduce competition depends on the rate at which amino acids become available in the soil solution and hence to what extent the two preceding steps (increased access to protein–phenolic complexes; depolymerization of proteins) are rate-limiting. It is therefore necessary to assess the mycorrhizal role in those two steps.

Lindahl et al. (2007) showed an increased C : N ratio in deeper humus layers, and this effect was attributed to selective N mining by ectomycorrhizal fungi. Several studies have provided explicit support that ectomycorrhizal fungi can mine humus layers for N and have identified the relevant ectomycorrhizal fungi (Hobbie et al., 2013; Rineau et al., 2013; Bödeker et al., 2014). Wu (2011) on the other hand claimed that direct access by ectomycorrhizal fungi to N from the protein–polyphenol complex is likely limited and attributed a major role for interactions between saprotrophic and ectomycorrhizal fungi. Current evidence suggests that arbuscular mycorrhizal fungi have neither the ability to degrade humus for N-rich compounds nor the ability to depolymerize proteins into amino acids. The widespread ability of arbuscular mycorrhizal fungi to take up amino acids may therefore not be related to closed nutrient cycles with a major role for uptake of organic nutrients, but may rather function as a scavenging mechanism to reabsorb exudates, including amino acids. More information about the role of arbuscular mycorrhiza in the uptake of organic N is provided in recent reviews by Veresoglou et al. (2012) and Hodge and Storer (2014).

The stable isotope  $^{15}\text{N}$  has been used to study the role of mycorrhizal symbioses in accessing different N pools. Whereas early studies had examined the congruence between the  $^{15}\text{N}$  signal of a potential N source and that of mycorrhizal fungi as evidence for uptake from that source, recent studies have emphasized the importance of N partitioning between fungus and plant (fractionation of N-depleted chitin or enriched proteins that are transferred to the plant) as a major control of isotopic composition (Hobbie and Högberg, 2012). Both the ability to take up N from organic sources (proteolytic fungi) and a relatively large transfer from fungus to plant are consistent with  $^{15}\text{N}$  enrichment of ectomycorrhizal fungi. Both mechanisms are likely correlated as fungi in more N-limited sites transfer relatively more N per unit C at the symbiotic interface. Further study of both traits is needed to better understand ectomycorrhizal fungal isotopic signatures, and especially cases of extreme enrichment (up to 20‰) where the nature of the N source is unknown.

A corollary of the conceptual model of Phillips et al. (2013) and of earlier models is that arbuscular mycorrhizal and ectomycorrhizal plants differ in their carbon and nutrient cycling traits (decomposability and nutrient release). Data by Cornelissen et al. (2001) were consistent with that prediction, showing that the mycorrhizal trait is a predictor for the so-called “fast–slow” spectrum (Reich, 2014). However, the comparison involved plant species that are not only different with regard to the mycorrhizal trait but also with regard to a number of other traits. Koele et al. (2012) applied phylogenetic correction, by comparing sister clades that differed only in their mycorrhizal habit. Their data, based on 17 pairs of taxa, indicate no differences in leaf N or phosphorus status after phylogenetic correction and imply that the mycorrhizal trait is correlated rather than causally related with these functional differences. Other claims about differences in N cycling between arbuscular mycorrhizal and ectomycorrhizal forests in the northern temperate zone may similarly indicate problems of establishing whether mycorrhizal status is a causally relevant or only a correlated trait. Thomas et al. (2010) showed a larger positive response to N deposition by arbuscular mycorrhizal than ectomycorrhizal trees, suggesting that the ability of the latter group to acquire organic N was

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traded off against the possibility of benefitting from increased inorganic N. Midgley and Phillips (2014) reported higher  $\text{NO}_3^-$  leaching in arbuscular mycorrhizal forests than in ectomycorrhizal forests, but as most of the data on arbuscular mycorrhizal forests pertain to maple (*Acer saccharum*) forests, the generality of that pattern needs further study.

Averill et al. (2014) reported that competition between ectomycorrhizal fungi/plants and decomposer microbiota results in N-limitation for the latter group, which retards litter breakdown and hence results in increased C storage. They noted 70% more C storage per unit N in ectomycorrhizal forests than in forests dominated by arbuscular mycorrhizal trees and suggested that mycorrhizal status exerts a much larger control over soil C than climatic variables at the global scale. However, this effect appears to be mainly driven by boreal trees (there is a dominance in the database of ectomycorrhizal trees belonging to the Pinales and Fagales, both orders that are characteristic for nutrient-poor soils) and the effect is only marginally significant when the analysis is performed on temperate and tropical forests (Averill et al., 2014). Therefore, plant traits that are inherently associated to mycorrhizal status should further be considered when assessing the key drivers of the differential C : N stoichiometry and C storage.

Nitrogen immobilization in the mycorrhizal mycelium may also have a large impact on the N cycle by reducing mineral N availability for plants. The general claim that mycorrhizal symbioses are beneficial for the plant and that cases of a negative plant performance in the mycorrhizal condition are explained by C costs of the symbiosis was refuted by Côrrea et al. (2012), who concluded that smaller plant size was caused by lower N uptake. Lower N content of the ectomycorrhizal plant could be due to mycorrhiza-driven progressive N limitation (Luo et al., 2004). Alberton et al. (2007) showed this to be the case as plant N content was significantly negatively correlated with hyphal length. Näsholm et al. (2013) showed that immobilization of N in the ectomycorrhizal mycelium can aggravate plant N limitation. They modelled competition between plant and fungus for N in a market model, and concluded that at N limitation the symbiosis does not alleviate plant N limitation but in fact even reduces plant

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growth (Franklin et al., 2014; Kuyper and Kiers, 2014). Yet, despite this negative effect on plant performance, a non-mycorrhizal strategy is competitively inferior, and therefore trees are trapped as they cannot terminate the association. Because the biomass of the arbuscular mycelium is usually one or two orders of magnitude smaller than that of the ectomycorrhizal mycelium, the amount of N immobilized by the arbuscular mycorrhizal mycelium is sometimes hypothesized to be quantitatively unimportant from the plant's perspective. However, recent studies (Hodge and Fitter, 2010; Grman and Robinson, 2013) indicate that N uptake and immobilization by arbuscular mycorrhizal fungi can also reduce plant performance.

Other pathways through which the mycorrhizal symbiosis may affect soil N cycling are modification of root exudation, root architecture, and fine root turnover (Churchland and Grayston, 2014). It is important to determine which of these differences are caused by the symbiosis and which by other root trait differences among species. For example, Comas et al. (2014) found that, after accounting for phylogenetic signals, ectomycorrhizal plants have thinner roots and greater branching intensity than arbuscular mycorrhizal plants.

In conclusion, it is still a matter of debate whether differences with respect to the mycorrhiza-associated nutrient economy (Phillips et al., 2013) are controlled by the mycorrhizal trait, or whether the mycorrhizal trait is instead correlated with causally relevant plant and climate traits. This needs to be resolved in the future.

#### 4 <sup>15</sup>N tracing modelling for understanding N cycling processes

The <sup>15</sup>N enrichment techniques for investigating gross N transformation rates have recently been reviewed (Rütting et al., 2011b; Huygens et al., 2013). Therefore, this section will focus on how these techniques, combined with modelling, have helped advance our understanding of N cycling dynamics in soils.

The stable isotope <sup>15</sup>N has been used as a tracer for the quantification of gross N transformation rates for 60 years. In their two seminal papers Kirkham and

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Bartholomew (1954, 1955) developed the isotope pool dilution technique, enabling for the first time the quantification of gross transformation rates of N cycling processes. Quantification of gross rates has deepened our understanding of the terrestrial N cycle tremendously. For example, Davidson et al. (1992) showed that old-growth forests exhibit high gross mineralization rates, challenging the paradigm (based on net mineralization rate measurements) that these ecosystems have low mineralization activity. The isotope pool dilution technique is still widely used, even though it has some important limitations. The most crucial disadvantage is that only total production and consumption rates of a labelled N pool can be quantified, which may be the result of several simultaneously occurring N processes (Schimel, 1996). For example, gross nitrification as quantified by the isotope pool dilution technique can be comprised of two separate processes, autotrophic ( $\text{NH}_4^+$  oxidation) and heterotrophic (the oxidation of organic N to  $\text{NO}_3^-$ ) nitrification. To overcome this limitation,  $^{15}\text{N}$  labelling can be done in conjunction with numerical  $^{15}\text{N}$  tracing models (Rütting et al., 2011b). These models describe the flow of N and  $^{15}\text{N}$  through the various soil N pools (e.g.  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and organic N), whereby transformations are represented by kinetic equations (e.g. zero- or first-order kinetics). The first  $^{15}\text{N}$  tracing model which could separate autotrophic from heterotrophic nitrification was presented by Myrold and Tiedje (1986). Subsequent studies using  $^{15}\text{N}$  tracing models have shown that heterotrophic nitrification can be a significant or even the dominant  $\text{NO}_3^-$  production pathway in forest and grassland soils (Barraclough and Puri, 1995; Rütting et al., 2008). In addition,  $^{15}\text{N}$  tracing models have been shown to be useful for investigating the importance of DNRA in various soils (Rütting et al., 2011a). Moreover, they can be used to distinguish DNRA from alternative pathways such as remineralization and plant efflux (Burger and Jackson, 2004). Recently an  $^{15}\text{N}$  amino acid pool dilution approach has been developed (Wanek et al., 2010), which can be a useful tool for investigating whether depolymerization or N mineralization is the rate limiting step of the terrestrial N cycle (Schimel and Bennett, 2004), particularly if incorporated in numerical  $^{15}\text{N}$  tracing models.

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In addition to quantification of gross N transformation rates,  $^{15}\text{N}$  enrichment has proven useful for partitioning nitrous oxide ( $\text{N}_2\text{O}$ ) emission sources. Using a two-source mixing model, Stevens et al. (1997) investigated the contribution of  $\text{NO}_3^-$  reduction (i.e. denitrification) and  $\text{NH}_4^+$  oxidation (i.e. autotrophic nitrification) to  $\text{N}_2\text{O}$  emission. Subsequent work, however, suggested that organic N can be a third substrate for  $\text{N}_2\text{O}$  production. Indeed,  $^{15}\text{N}$  studies using a triplet tracer approach and either analytical (Stange et al., 2009) or numerical (Stange et al., 2013; Müller et al., 2014)  $^{15}\text{N}$  tracing models showed a significant or even dominant contribution of oxidation of organic N (heterotrophic nitrification) to  $\text{N}_2\text{O}$  production in soils. The numerical models have the additional advantage that gross  $\text{N}_2\text{O}$  production rates can be quantified. Using oxygen isotopes ( $^{18}\text{O}$ ) as an additional tracer allows the separation of  $\text{NH}_4^+$  derived  $\text{N}_2\text{O}$  emission between  $\text{NH}_4^+$  oxidation and nitrifier-denitrification (see Sect. 2.2). A further step for understanding sources of  $\text{N}_2\text{O}$  emission from soil would be to incorporate  $^{18}\text{O}$  into numerical tracing models, i.e. development of a combined  $^{15}\text{N}$ - $^{18}\text{O}$ -tracer model. Overall, stable isotope labeling approaches ( $^{15}\text{N}$  and  $^{18}\text{O}$ ) have greatly increased our understanding of the diverse N cycle processes contributing to  $\text{N}_2\text{O}$  production in soils. Moreover, these studies have confirmed the importance of  $\text{NO}_2^-$  dynamics for  $\text{N}_2\text{O}$  production (Stange et al., 2013; Müller et al., 2014) and for the soil N cycle in general (Rütting and Müller, 2008; Isobe et al., 2012), which deserves attention in future studies.

## 5 Conclusions

This is an exciting time to study the soil N cycle. Years of surprising findings on unanticipated pathways and mechanisms have expanded the horizons of researchers. These findings have stimulated efforts to develop and test new methods for quantifying these processes. This has resulted in a better understanding of the complexity of soil N cycling processes as well as powerful tools for future exploration.

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Critical challenges remain. Many processes are still difficult to quantify and variability and heterogeneity hampers our ability to provide well constrained estimates relevant to water and air quality issues. We postulate that addressing the questions formulated above would constitute a comprehensive research agenda with respect to the N cycle for the next decade. Success will require interactions between soil science and other disciplines that address both smaller (e.g., molecular and microbial) and larger (ecosystems, landscapes and regions) scales. Such an agenda would help us meet future challenges on food and energy security, biodiversity conservation as well as climate stability.

*Author contributions.* All authors contributed to selecting the topics addressed in this manuscript. P. Boeckx wrote the sections on BNF and N<sub>2</sub>O consumption; T. Rütting wrote the section on <sup>15</sup>N models; D. Huygens and T. W. Kuyper co-wrote the section on mycorrhizal associations; D. Huygens wrote the section on rhizodeposition and plant traits; I. M. Lubbers and J. W. van Groenigen co-wrote the section on soil fauna; J. W. van Groenigen wrote the section on nitrifier denitrification; P. M. Groffman wrote the section on denitrification. J. W. van Groenigen, D. Huygens and P. M. Groffman co-wrote the remaining sections. All authors commented on the final draft.

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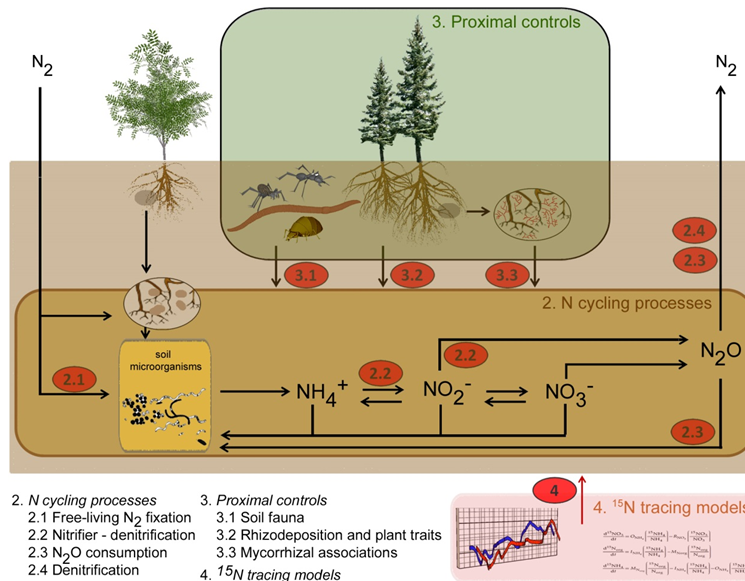
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- 2. *N* cycling processes
  - 2.1 Free-living  $N_2$  fixation
  - 2.2 Nitrifier - denitrification
  - 2.3  $N_2O$  consumption
  - 2.4 Denitrification
- 3. *Proximal controls*
  - 3.1 Soil fauna
  - 3.2 Rhizodeposition and plant traits
  - 3.3 Mycorrhizal associations
- 4.  $^{15}N$  tracing models

**Figure 1.** New insights and key challenges with respect to the soil N cycle, as identified in this manuscript. These include four N cycling processes (Sects. 2.1–2.4), three proximal controls on N cycling processes (Sects. 3.1–3.3), and a modelling challenge (Sect. 4).

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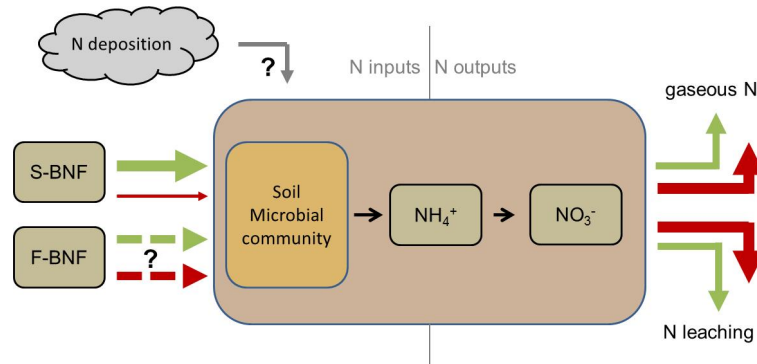
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**Figure 2.** The “leaky nitrostat” model adapted from Hedin et al. (2009), indicating the importance of symbiotic (S-BNF) and free-living (F-BNF) biological N<sub>2</sub> fixation along a forest successional gradient, from young (green) to mature (red) forest stands. At the initial stages of ecosystem succession, the N supply via S-BNF, F-BNF and N deposition supports high ecosystem N demands. In mature forest stands with a lower N demand, S-BNF is down-regulated, but N inputs via F-BNF and N deposition lead to ecosystem N losses via N leaching and gaseous N production.

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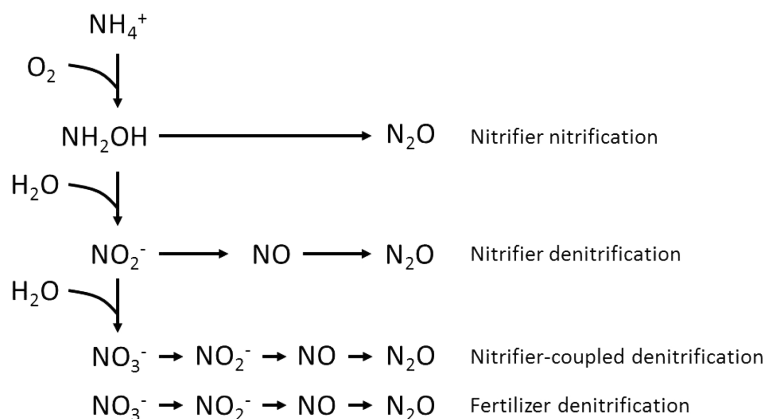
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**Figure 3.** Different pathways of  $\text{N}_2\text{O}$  production in soil. Classical nitrification by autotrophic bacteria or archaea (nitrifier nitrification); nitrifier denitrification by the same group of autotrophic bacteria; nitrification followed by denitrification (nitrification-coupled denitrification) and denitrification of applied nitrogen fertilizer (fertilizer denitrification). Reproduced from Kool et al. (2011a).

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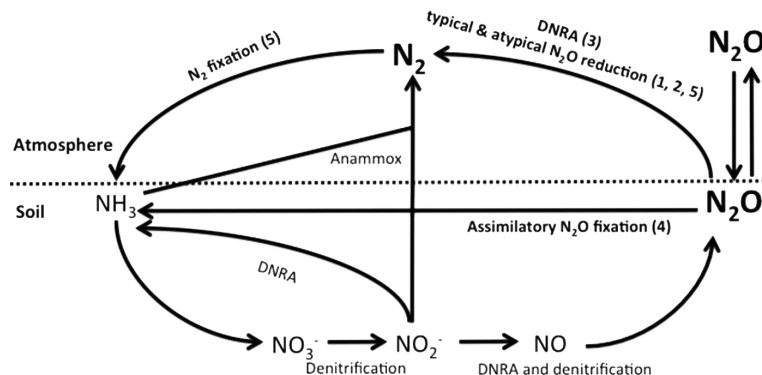
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**Figure 4.** The  $N_2O$  production and consumption network showing five pathways for  $N_2O$  consumption. Dissimilatory  $N_2O$  reduction to  $N_2$  via typical, denitrifier nosZ I (1), atypical, non-denitrifier nosZ II (2), dissimilatory  $NO_3^-$  reduction to  $NH_3$  (DNRA) (3), direct assimilatory  $N_2O$  fixation via nitrogenase to  $NH_3$  (4), and indirect assimilatory  $N_2O$  fixation ( $N_2O$  reduction to  $N_2$  followed by  $N_2$  fixation) (5); abiotic pathways that produce gaseous N (Feammox and chemo-denitrification are not shown).

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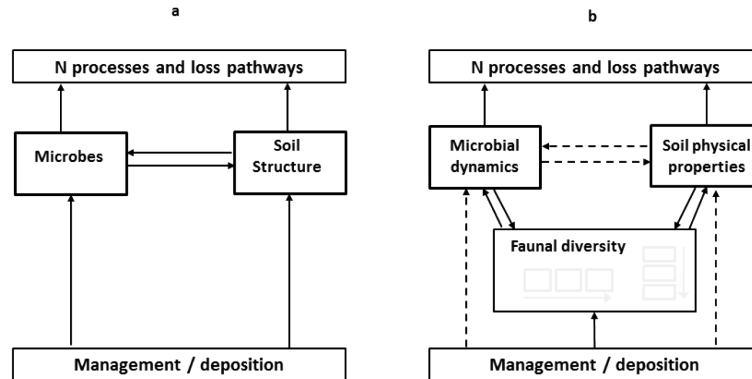
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**Figure 5.** The influence of soil fauna on soil N processes and loss pathways. Conventionally, these processes and loss pathways were often considered as the result of interactions between microbes and soil structure **(a)**. More recently, it is recognized that many microbial and physical properties are influenced by faunal diversity through trophic relations and through changes in the soil structure by ecosystem engineers **(b)**.

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