MINIREVIEW

Nitrogen turnover in soil and global change

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Abstract

Nitrogen management in soils has been considered as key to the sustainable use of terrestrial ecosystems and a protection of major ecosystem services. However, the microorganisms driving processes like nitrification, denitrification, N-fixation and mineralization are highly influenced by changing climatic conditions, intensification of agriculture and the application of new chemicals to a so far unknown extent. In this review, the current knowledge concerning the influence of selected scenarios of global change on the abundance, diversity and activity of microorganisms involved in nitrogen turnover, notably in agricultural and grassland soils, is summarized and linked to the corresponding processes. In this context, data are presented on nitrogen-cycling processes and the corresponding microbial key players during ecosystem development and changes in functional diversity patterns during shifts in land use. Furthermore, the impact of increased temperature, carbon dioxide and changes in precipitation regimes on microbial nitrogen turnover is discussed. Finally, some examples of the effects of pesticides and antibiotics after application to soil for selected processes of nitrogen transformation are also shown.

Introduction

Nitrogen is one of the crucial nutrients for all organisms (LaBauer & Treseder, 2008), as it is an essential component of important biopolymers. However, most of the N in nature occurs as dinitrogen gas or is fixed in organic compounds, like proteins or chitin, both of which cannot be directly used by plants and animals. Only specialized microorganisms are able to transform the gaseous dinitrogen into ammonia or to make organically bound N bioavailable by mineralization. Not surprisingly, N input by fertilization has always been a key factor for high crop yields and plant quality. Therefore, crop production is by far the single largest cause of human alteration of the global N cycle (Smil, 1999). Whereas in preindustrial times exclusively organic fertilizers had been used, the invention of the Haber Bosch procedure in the 20th century made huge amounts of mineral fertilizer available. The doubling of world food production in the past four decades could only be achieved with a strong land-use intensification including an almost sevenfold increase of N fertilization (Tilman, 1999) as well as wide-ranging land reclamations. These developments have contributed to the doubling of N loads to soil since the beginning of the 20th century (Green *et al.*, 2004). The total global N input in the year 2000 was about 150 TgN (Schlesinger, 2009), whereas supply in croplands via mineral fertilizer was the single largest source accounting for almost half of it. Surprisingly, N entry from N-fixation was the second largest factor and contributed to 16%, while manure and recycled crop residues provided similar amounts and each accounted for only 8–13% of the total global supply. Remarkably, the entry of N via atmospheric deposition was in the same range. In regions with high mineral fertilizer application, the highest N accumulation potential in ecosystems could be observed, whereas the accumulation of N leads to high impacts on environmental quality like loss of diversity (Cragg & Bardgett, 2001), dominance of weed species (Csizinszky & Gilreath, 1987) and soil acidification (Noble *et al.*, 2008). Additionally, land-use intensification also results in an increased use of bioactive chemicals, like pesticides and herbicides as well as antibiotics, which enter the environment via manure (Lamshöft *et al.*, 2007).

According to Liu *et al.* (2010), 55% of the global applied N was taken up by crops. The remainder was lost in leaching

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(16%), soil erosion (15%) and gaseous emission (14%). Such N depletion of soils leads to eutrophication (Stoate *et al.*, 2009), surface- and groundwater pollution (Spalding & Exner, 1993) and emission of the greenhouse and ozone-depleting gas nitrous oxide (N_2O) (Davidson *et al.*, 2000), impacting on human health and climate change (Fig. 1).

To reduce these threats, Schlesinger (2009) suggested that policy makers and scientists should focus on increasing N-use efficiency in fertilization, reducing transport of reactive N fractions to rivers and groundwater and maximizing denitrification to N_2.

Because of the use of advanced molecular tools (Gabriel, 2010) and stable isotopes (Baggs, 2008) in recent years, scientists have been able to identify new key players of N turnover for selected processes like nitrification (Leininger *et al.*, 2006) or N-fixation (Chowdhury *et al.*, 2009) as well as completely new processes like anammox (Op den Camp *et al.*, 2006). All these findings have revolutionized our view of N transformation processes in soils, although the relevance for the overall understanding of N transformation is not entirely clear yet and discussed controversially in the literature. However, despite numerous studies and a large amount of collected data, we have to admit that N turnover and factors driving the corresponding populations are not yet completely understood.

Furthermore, according to the *UN Millenium Ecosystem Assessment* (http://www.maweb.org/), global change will highly affect N turnover in soils to a so far unknown extent. According to the definition given in Wikipedia, the term 'global change' encompasses interlinked activities related to population, climate, the economy, resource use, energy development, transport, communication, land use and land cover, urbanization, globalization, atmospheric circulation, ocean circulation, the C cycle, the N cycle, the water cycle and other cycles, sea ice loss, sea-level rise, food webs, biological diversity, pollution, health, overfishing and alteration of environmental conditions including climate change as well as land-use changes and effects of xenobiotic substances. Therefore, there is a need for experimental approaches to study the consequences of altering environmental conditions including climate change as well as land-use changes and the effects of xenobiotic substances on N turnover in soil. In the following review, state-of-the-art knowledge is summarized concerning the impact of selected global change scenarios on microbial N turnover as well as the abundance and diversity of key players. Additionally, implications for future research strategies and priorities are given.

Ecosystem development

Natural and anthropogenic activities lead to new terrain for soil development. In this context, different chronosequences of ecosystem development like glacier forefields, sand dunes, volcanoes or restoration sites have emerged. These are interesting aspects to study the development of N-cycling processes as well as the contributing functional microbial groups. Overall, three phases can be postulated: initial, intermediate and mature phases. Depending on the investigated ecosystem, these phases can range from a few days or weeks (Jackson, 2003) to hundreds of years (Kandeler *et al.*, 2006; Brankatschk *et al.*, 2011), respectively.

Most of the initial ecosystems are characterized by nutrient shortage, barren substrate and scarce vegetation (Crews *et al.*, 2001; Nemergut *et al.*, 2007; Smith & Ogram, 2008; Lazzaro *et al.*, 2009; Brankatschk *et al.*, 2011). The total N concentrations are often far below 0.1% and only traces of ammonia and nitrate can be measured (Brankatschk *et al.*, 2011). Additional N input by the weathering of bedrock material is unlikely as it only contains traces of N. Thus, the colonization with N-fixing microorganisms seems to be the only way for N input, despite the high energy demands for the transformation of N_2 into ammonium. Crews *et al.* (2001) demonstrated that the total N input in young lava flows was mainly driven by N fixation, although fixation rates were low. This has been confirmed in several other studies, which demonstrated a high abundance of nonheterocystous N-fixing cyanobacteria like *Microcoleus vaginatus* (Yeager *et al.*, 2004; Nemergut *et al.*, 2007; Abed *et al.*, 2010). It is obvious that in initial ecosystems, cyanobacteria play a prominent role in ecosystem engineering. They not only improve the N status of soils by N-fixation, but also secrete a polysaccharide sheath, resulting in the formation of soil crusts. This leads to a stabilization of substrates, capture of nutrients and an increase of the water-holding capacity, which paves the way for other organisms and processes (Garcia-Pichel *et al.*, 2001; Schmidt *et al.*, 2008). Therefore, at early stages of soil development heterotrophic microorganisms, which are able to mineralize the N derived from air-driven deposition (e.g. chitin) or ancient and recalcitrant materials are able to find their niches and stimulate N turnover (Bardgett *et al.*, 2007; Brankatschk *et al.*, 2011). However, this process is highly energy demanding and thus the turnover rates typically low. Obviously, as only limited competition for N resources exists at this stage (due to a lack of plants), the amount of ammonia is sufficient for the development of microbial communities involved in nitrification. This process results in the formation of nitrate, which leaves the ecosystem mainly by leaching. Therefore, N accumulation rates at initial sites are low (Tscherko *et al.*, 2004).

If the total N concentrations in soil exceed 0.2%, plant development starts and cyanobacterial soil crusts are displaced by shadowing by plant growth (Brankatschk *et al.*, 2011). Therefore, the intermediate stage of ecosystem development is characterized by increasing plant coverage and surface stabilization resulting in an increased C input

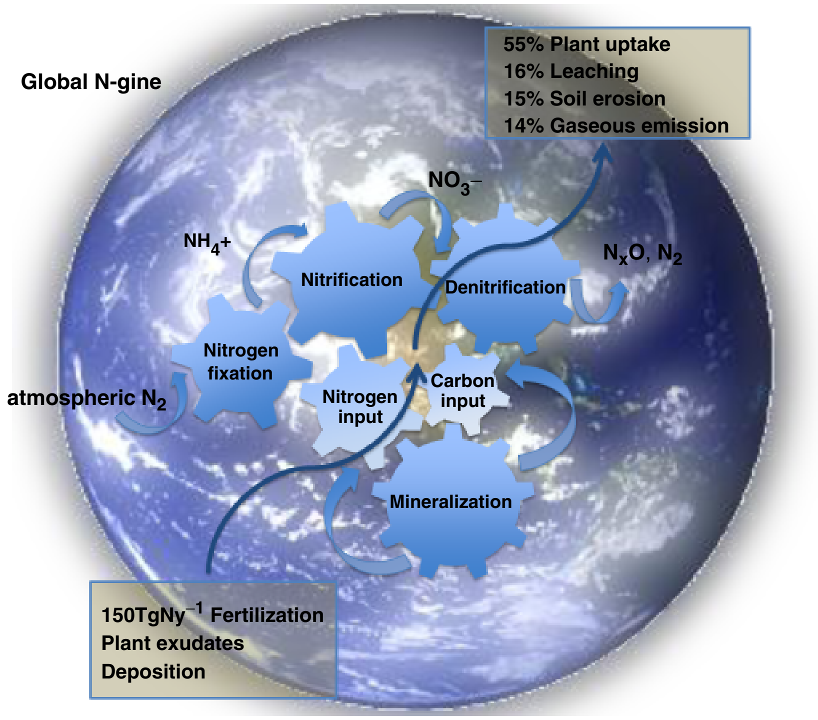

Fig. 1. Nitrogen turnover at the global scale.

via exudation and litter material. However, ammonium and nitrate contents are still much lower (Kandeler *et al.*, 2006; Brankatschk *et al.*, 2011) than in well-developed grassland sites (Chronáková *et al.*, 2009). Although it has been argued that this stage of ecosystem development is characterized by a competition between microorganisms and plants for N (Schimel & Bennett, 2004; Hämmerli *et al.*, 2007), associative or symbiotic networks between N-fixing microorganisms (mainly bacteria) and plants become a central element at this stage (Duc *et al.*, 2009). This results in an increased N-fixation activity in the rhizosphere and a highly efficient share of nutrients between plants and microorganisms. Because of the patchy distribution of C and N concentrations at those sites, many studies have revealed the highest microbial diversity at intermediate stages of ecosystem development by targeting functional genes like *nifH* (Duc *et al.*, 2009) or general microbial diversity by 16S rRNA gene (Gomez-Alvarez *et al.*, 2007). This fits with the intermediate-disturbance hypothesis, postulating that medium disturbance events cause the highest diversification (Molino & Sabatier, 2001). However, besides the development of plant–microorganism interactions, the intermediate phase of ecosystem development is also characterized by highly efficient degradation of litter and subsequent N mineralization (Esperschütz *et al.*, 2011) as well as an increase in fungal biomass (Bardgett & Walker, 2004), probably also of arbuscular mycorrhiza, which may contribute to a better distribution of the N in soil with ongoing succession. At this stage, the abundance and activity of nitrifiers (Nicol *et al.*, 2005) and denitrifiers (Smith & Ogram, 2008) is still low due to the high N demand of the plants. Whether typical plants at those sites are able to produce nitrification inhibitors to better compete for ammonium might be a highly interesting question for future research (Verhagen *et al.*, 1995).

In contrast, when total N concentrations above 0.7% are reached in soils at well-developed sites and vegetation is no longer dominated by legumes, nitrification becomes a highly significant process. Interestingly, in ecosystems of glacier forefields, nitrification activity seems to be driven by ammonia-oxidizing archaea (AOA), although being lower in abundance than their bacterial counterpart [ammonia-oxidizing bacteria (AOB)]. This might be due to the better adaptation to relative ammonium-poor environments (Di *et al.*, 2009) and low pH (Nicol *et al.*, 2008). In combination with pronounced root penetration resulting in increased exudation, enhanced water retention potential and less oxygen diffusion (Deiglmayr *et al.*, 2006), denitrification becomes a key process for the overall N budget at those sites in soil. Interestingly, Brankatschk *et al.* (2011) only found a good correlation of a part of the functional genes of the denitrification cascade, for example, *nosZ* (nitrous oxide reductase) gene abundance and potential denitrification activity, whereas *nirK* and *nirS* (nitrite reductases) gene abundance did not correlate with the rates of potential activity. Moreover, the highest relative gene abundance of *narG* was observed in early development stages of soils (Kandeler *et al.*, 2006), while the nitrate reductase activity peaked at late stages of soil development (Deiglmayr *et al.*,

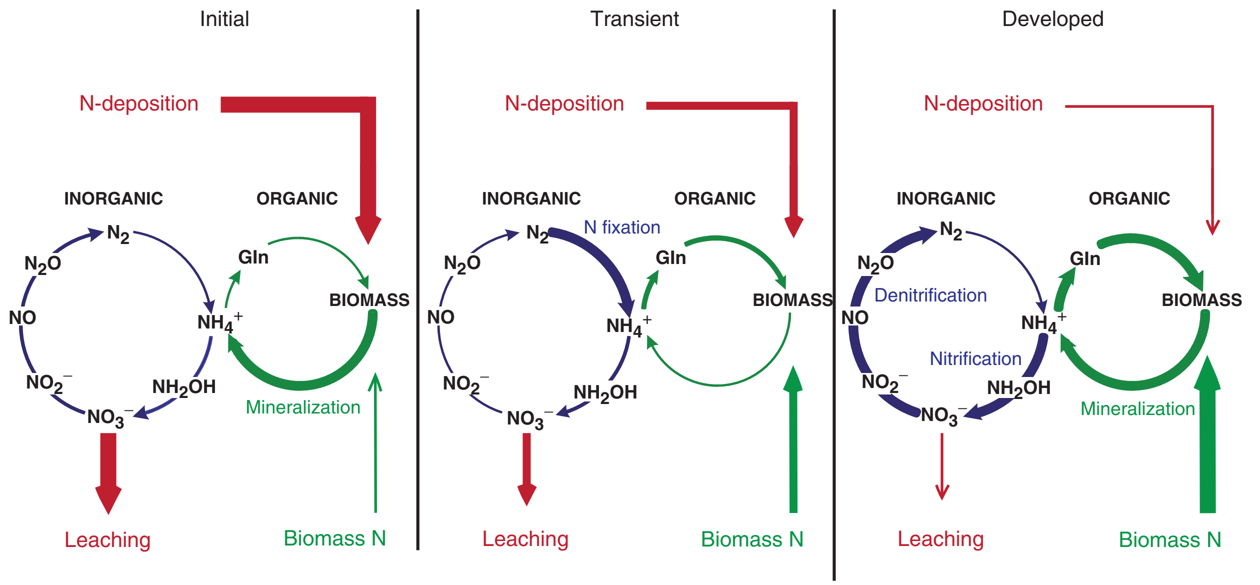

Fig. 2. Scheme of the development of the nitrogen cycle during ecosystem development (initial, transient and developed). The size of the arrows represents the impact of the corresponding process for nitrogen turnover.

2006). Similar observations were made by Smith & Ogram (2008) along a restoration chronosequence in the Everglades National Park. The mechanistic bases for these observations are still not clear. In addition to high activities of nitrifiers and denitrifiers at well-developed sites, the highest values of mineralization activity have been observed there in several studies (Tscherko *et al.*, 2004; Brankatschk *et al.*, 2011). These data are congruent with the observations of Frank *et al.* (2000), who found a positive correlation between nitrification, denitrification and N mineralization processes in Yellowstone Park grasslands.

Overall, the studies performed so far using the chronosequence approach to describe ecosystem development have revealed surprisingly similar patterns of the participation of different functional groups of microorganisms involved in N cycling at the three different phases (Fig. 2). In summary, all systems described were characterized by very low C and N concentrations in soil as well as less pronounced organismic networks of interaction at the initial stages of soil development.

Changing land-use patterns

A generalization of the results described above to other scenarios of global change related to ecosystem development, for example, in response to natural disasters (earthquakes), after manmade destructions (clear cuts of forest sites) or due to land-use changes is not possible. This is due to the different quality and amount of C and N present in soil as well as the biodiversity, mainly related to soil animals and plants at the initial stages in these disturbed systems. Whereas the consequences of natural disasters for N turnover have been rarely addressed, the impact of land-use changes on N turnover and the corresponding functional communities has been studied extensively. However, in this context, it is difficult to identify *one* main driver for shifts in the microbial population structure, as land-use changes often encompass a combination of different forms of management. For example, the use of extensively used grassland for crop production will not only change aboveground biodiversity, but will also result in changes in pesticide application, tillage and fertilizer management.

Overall, the conversion of forests or grasslands to agricultural land has an impact on almost all soil organisms (Postma-Blaauw *et al.*, 2010). Therefore, the functional diversity of microorganisms involved in N cycling is also highly influenced by land-use changes. This has been well documented for nitrifiers and denitrifiers, whereas surprisingly for N-fixing bacteria, clear response patterns have been described in only a small number of cases. In some cases, even no response of *nifH* towards land-use changes was detected (Colloff *et al.*, 2008; Hayden *et al.*, 2010), which might be related to the high concentrations of ammonium and nitrate before land-use change. In terms of nitrification, good correlations between gene abundance and land use have been described for AOB in several studies. Colloff *et al.* (2008) found higher gene abundance of the bacterial *amoA* gene in agricultural soils compared with soils from rainforests. By contrast, Berthrong *et al.* (2009) observed consistently reduced nitrification rates in soils that were converted from grassland into forest. These trends were also confirmed by Bru *et al.* (2010) comparing land-use changes between forests, grassland and agricultural soils in different parts of the world. The authors found a strong correlation between AOB and the form of land use. Interestingly, in the same study, no differences were observed for archaeal ammonia oxidizers (AOA) in relation to the investigated land-use types. Hayden *et al.* (2010) almost consistently observed a greater abundance of AOB *amoA* genes in managed compared with remnant sites. The good correlation between AOB and land use might be related to the different ammonium concentrations in soil in response to different land-use types. AOB often colonizes habitats with high ammonium concentrations, whereas for AOA abundance, so far, no general dependency on ambient ammonium concentration has been documented. Furthermore, the results might be related to the high sensitivity of AOB towards low pH, which is often present in forest soils and leads to low availability of ammonia.

It was reported that land-use changes from forest to grassland soils are often accompanied by high N losses from soil (reviewed by Murty *et al.*, 2002). However, no clear trends are visible so far, if these losses occur in general due to increased denitrification rates or leaching of the nitrate formed during nitrification, as both observations have been described in the literature. This might be explained by the different soil types under investigation in the various studies. Whereas in loamy soils, which tend to have more anoxic microsites, denitrification might be stimulated (Rich *et al.*, 2003; Boyle *et al.*, 2006), in sandy soils, the nitrate formed may leach fast to the groundwater (Murty *et al.*, 2002). For denitrifiers, land-use changes overall influence the abundance and diversity patterns of selected functional groups. Attard *et al.* (2010) described, for example, higher potential denitrification rates in grassland soils compared with soils under cropping management. This was in accordance with a 1.5–5-fold higher abundance of denitrifiers (based on the abundance of *nirK* genes) in grassland soils than arable soils found in various studies (Baudoin *et al.*, 2009; Attard *et al.*, 2010), including shifts in the diversity patterns of *nirK*-harboring bacteria. Whereas a strong correlation between gene copy numbers of *nirK* and potential denitrification rates has been described, no correlation was found between the diversity patterns of *nirK* and turnover of nitrate. This indicates highly similar ecophysiological patterns of nitrite reducers of the *nirK* type.

Agricultural management

Not only changes in land-use patterns, but also shifts in agricultural management practice can result in alterations of functional microbial communities involved in N cycling. In general, there is consensus that an intensification of agriculture and subsequent increased fertilization regimes result in higher nitrification and denitrification rates as well as an increase of both functional groups (Le Roux *et al.*, 2003, 2008; Patra *et al.*, 2006). In the case of ammonia oxidizers, mainly AOB benefit from the increased availability of ammonium in soil (Schauss *et al.*, 2009a). For N-fixing prokaryotes, several studies have indicated a reduction based on the abundance of *nifH* and consequently also lower N-fixation activity in highly fertilized soils (Coelho *et al.*, 2009). Interestingly, the inoculation of seeds from legumes with rhizobia, which is a common practice in low-input farming to enhance N-fixation, does not only increase *nifH* abundance in the rhizosphere, but also leads to higher abundance of nitrifiers and denitrifiers (Babic *et al.*, 2008). This indicates that at least a part of the fixed N is released into soil, despite the symbiotic interaction (Babic *et al.*, 2008). As the use of monocultures and the intensification of agriculture *per se* (including the transformation of sites, which are less suited for agriculture, for the production of renewable resources) is often accompanied by a loss in nutrients (Malézieux *et al.*, 2009), which is primarily compensated by the application of inorganic fertilizers, changes in N turnover and the corresponding microbial communities might be primarily a result of changed fertilization regimes, as described by Drury *et al.* (2008). It has been confirmed in several studies that the type of fertilizer (mineral vs. organic fertilizer) has a clear influence on the N budget of soils and the corresponding functional microbial groups (Hai *et al.*, 2009; Ramirez *et al.*, 2010). As expected, the application of a mineral fertilizer based on ammonia-nitrate increases the nitrification and denitrification patterns in soil shortly after application, when the fertilizer is not taken up by the plant due to increased availability of the corresponding substrates. In contrast, the application of an organic fertilizer leads to higher abundance of microorganisms involved in mineralization and only relatively slight increases of nitrifiers and denitrifiers and their activity in the long run. Because of the overall more balanced N budget in soil when organic fertilizers are applied, N-fixing microorganisms are favored by this practice (Pariona-Llanos *et al.*, 2010). Not surprisingly, the effects observed in soils that have been used for grazing can be compared with those where manure has been applied, including clear shifts mainly in the diversity patterns of ammonia- and nitrite-oxidizing microorganisms as well as denitrifiers (Chroňáková *et al.*, 2009) Furthermore, grazing also induces shifts in root exudation patterns (Hamilton & Frank, 2001), which may further influence the abundance and activity of microorganisms involved in N turnover.

In the last decades, the influence of tillage management on N turnover has been studied in several projects, as nontillage systems have been described to be of advantage in terms of nutrient supply and are very popular in organic farming (Hansen *et al.*, 2011). Overall, changes in nitrification activity after modifying the tillage practice were well explained by the accumulation of ammonium in the top soil due to nontillage and the corresponding changes in the abundance of nitrifiers (Attard *et al.*, 2010). In most studies, performed so far, a higher nitrification activity and subsequent higher nitrate concentrations in soil were linked to increased denitrification rates in the top soil layer in nontillage compared with tillage treatments (Petersen *et al.*, 2008; Baudoin *et al.*, 2009; Attard *et al.*, 2010). This is due to tillage-induced higher C concentrations in top soils and a stronger formation of aggregates with anoxic microsites due to a lack of tillage-induced mixing. In addition, tillage results in a merging of the surface soil layers with the lower layers, the latter being characterized by lower denitrification potential (Attard *et al.*, 2010), which causes overall lower denitrification rates and abundance of the corresponding functional genes (especially *nirK*). However, as stated above, in most cases, changes in tillage management are accompanied by changes in pest management and cropping sequences. The changes observed in long-term studies therefore cannot be linked conclusively to tillage management alone. Thus, most studies performed so far in this area were linked to short-term perturbations. They may not reflect the typical response patterns of the soil microorganisms to the new conditions after the change of the tillage management, as they do not account for microbial adaptation, in the context of the intermediate-disturbance hypothesis (Molino & Sabatier, 2001) as well as the increasing C contents in the top soils over time where nontillage practice has been performed.

Changing climatic conditions

Because of ongoing climate change, various modifications in land use and agricultural management have been implemented. Thereby, climate and land management are highly interlinked and cannot be separated. In addition, it is well accepted that climatic conditions notably influence microbial performance in soil. Thus, several studies have been performed to estimate the consequences of increased atmospheric temperature or carbon dioxide (CO_2) concentrations as well as shifts in precipitation on N turnover and the corresponding functional communities.

In general, it is difficult to simulate increased temperature scenarios in experiments, as an increase of the average temperature of 3 °C over the next 50 years would at most result in an annual increase of < 0.2 °C. Therefore, experiments comparing soils with ambient temperature with soils increased in temperature by 2–5 °C do not simulate climate change, but are more appropriate to understand the overall stress response of the soil microbial community. An air temperature increase of 3 °C for example, induced shifts in the AOB community structure, decreased AOB richness and concurrently increased potential nitrification rates in the rhizosphere of legumes. It remains open whether AOA adopted the ability to transform ammonia, while their bacterial counterparts were sensitive to the elevated temperature (Malchair *et al.*, 2010a). Besides questioning the relevance for studying climate change effects, it is unclear whether the observed shifts were a direct effect of the temperature or were rather related to changes of the plant performance, for example, increased exudation, in response to the increased temperature.

More relevant in the context of temperature-related effects are questions addressing changes in soils of permafrost regions, as here, only a slight increase of air temperature results in a prolonged period in which soils are unfrozen during the summer time. In these studies, the focus has mostly been on C turnover and methane emission, although clear effects on N transformation have been described. There is broad agreement that thawing of permafrost soils leads to a rapid increase of denitrification and hence high N_2O emissions, due to the high water saturation and the availability of easily degradable C and nitrate in those soils (Repo *et al.*, 2009; Elberling *et al.*, 2010). Measured emissions were comparable to values from peat soils (0.9–1.4 g N_2O m^{-2} and year). In contrast, nitrifying communities did not benefit from the changed environmental conditions in the short run. Metagenomic analysis and clone library studies revealed a low diversity and a relatively low abundance for ammonia oxidizers (AOA and AOB) (Liebner *et al.*, 2008; Yergeau *et al.*, 2010). Obviously, the high concentrations of available C as well as the anoxic conditions do not favor the growth of AOA and AOB. Therefore, not surprisingly, in permafrost soils, clear evidence for anaerobic ammonia oxidation has been obtained (Humbert *et al.*, 2010), in contrast to many other soil ecosystems. N-fixing microorganisms did not play a major role in the investigated sites and did not change in abundance and diversity after thawing (Yergeau *et al.*, 2010).

However, also in moderate climatic zones, small shifts in the temperature affect freezing and thawing regimes in soil during winter time and increased numbers of freezing–thawing cycles are expected. Therefore, this topic is of interest for agricultural management practice, notably when intercropping systems are used over winter. Like in permafrost regions, soil thawing is mainly accompanied by an accelerated release of nutrients, but also by the emission of greenhouse gases, such as N_2O and nitric oxide (NO), as well as CO_2 and methane. Considerable research was focused on gaseous N losses and the N_2O/N_2 ratio in the last two decades (Philippot *et al.*, 2007). A modeling study by De Bruijn *et al.* (2009) indicated that N_2O emissions resulting from freezing–thawing are not monocausal and mainly depend on the amount and quality of available C and N, the microbial biomass and the redox conditions in soil after thawing. Although N_2O emissions were reported from soils that are generally characterized by a low temperature (< 15 °C), these values are far lower than the N_2O concentrations emitted from thawing soils (Koponen & Martikainen, 2004). Wolf *et al.* (2010) could show that up to 70% of the annual N_2O emissions from agricultural fields might occur in the winter period. Peak emissions of N_2O were reported from arable soils during or shortly after thawing (Dörsch *et al.*, 2004) and could only be attributed in part to N_2O physically trapped in soil aggregates during freezing (Teepe *et al.*, 2001). A large part of N_2O arises from the microbial denitrification process, which fits with decreased oxygen and increased C and N availabilities in soils that were subject to freezing–thawing cycles (Öquist *et al.*, 2004). Sharma *et al.* (2006) observed an increase in transcripts of the nitrate and nitrite reductase genes *napA* and *nirK*, respectively, straight after thawing began. Other studies have shown a significant increase in N mineralization compared with nonfrozen soils (De Luca *et al.*, 1992). In contrast to permafrost soils, where aerobic ammonium oxidation did not play an important role, increased nitrification rates were measured after thawing in soils from moderate climatic zones. Su *et al.* (2010) demonstrated that bacterial ammonia oxidizers were impaired by freezing and thawing, whereas their archaeal counterparts even increased in abundance. This is in accordance with the hypotheses by Schleper *et al.* (2005) and Valentine (2007), who presumed that archaea are more tolerant to stress conditions than bacteria. Therefore, archaea could be the main contributors to ammonia oxidation after freezing and thawing.

Studies on the effects of changes in precipitation on microbial N turnover are rare, notably when questions about the effects of extreme weather events are addressed, although it is well accepted that the increased variability in precipitation and the resulting soil water dynamics directly alter N cycling in terrestrial ecosystems (Corre *et al.*, 2002; Aranibar *et al.*, 2004). Not surprisingly, irrigation increased, on the one hand, nitrate leaching rates mainly in sandy soils (Olson *et al.*, 2009). On the other, increased denitrification activities were measured. For example, scenarios simulating high rainfall events resulted in 2.4–13-fold increases in ammonia, nitrate, NO and N_2O fluxes in clay loam, whereas NO and N_2O fluxes decreased in sandy soils in response to water drainage (Gu & Riley, 2010). Ruser *et al.* (2006) found maximum N_2O emission rates in differently compacted soils after rewetting of dry soil that increased with the amount of water added. Muhr *et al.* (2008) postulated that rather than the intensity of rewetting, the length of the drought period might be more important for the process patterns and the microbial communities involved in N_2O and NO emissions. Again, the effects of precipitation depend on other factors like agricultural management. For example, it could be shown that the effects of irrigation depend on the type of cover crop in soil (Kallenbach *et al.*, 2010). Overall, studies mainly focused on the effects of precipitation on denitrification rates. Other processes of the N cycle as well the corresponding communities have been rarely studied so far. It must also be assumed that these processes are also highly affected directly or indirectly by dryness and precipitation, respectively. Interestingly, Zavaleta *et al.* (2003) demonstrated changes in plant diversity patterns in different grasslands in response to different precipitation regimes, which may indicate indirect effects of different precipitation regimes on nitrifiers as well as on N-fixing microorganisms.

The same authors could show that enhanced CO_2 concentrations in the atmosphere decrease plant diversity at grassland sites. However, C input into the soil via exudation was enhanced, which resulted in an overall stimulation of most microorganisms. Mainly N-fixing bacteria benefited from the additional C input, as their abundance was increased at grassland sites with increased CO_2 (He *et al.*, 2010). As expected, enhanced CO_2 concentrations also stimulated denitrifiers in soil due to a general reduction of the redox potential in soil as a result of the increased microbial activity (Pinay *et al.*, 2007). Furthermore, a stimulation of N mineralization has been proven (Muller *et al.*, 2009). Consequently, elevated CO_2 values in the atmosphere resulted in reduced abundance of autotrophic microorganisms like ammonia oxidizers (Horz *et al.*, 2004) in combination with reduced activity patterns (Barnard *et al.*, 2006) due to competition from heterotrophs as well as lower and lower activity in grassland soils. In addition, several studies have described a positive correlation between plant species richness and AOB richness in grassland soils. Malchair *et al.* (2010b) hypothesized that this link could be due to the spatial heterogeneity of ammonia, promoted by the plant species richness. In contrast, AOB were unaffected by increased atmospheric CO_2 (Nelson *et al.*, 2010) in soils under intensive agricultural use (e.g. soybean or maize cultivation), probably as the present ecotypes in these soils are already adapted to higher C input into the soil, for example, by manuring, litter application and intensive exudation by the cultivated crop. However, when relating those results to ongoing climate change, it must be considered, as described above for temperature effects, that we are challenged with an continuous increase in CO_2 concentrations in the atmosphere and not with a doubling from 1 day to another as simulated in most experiments.

Xenobiotics

New climatic conditions and changed agricultural practice have led to an emerging pressure from weeds and phytopathogens, which complicates farming practice and has resulted in the increased use of (new) chemical substances worldwide. Pesticides, i.e. herbicides, fungicides and insecticides, can exert collateral effects on soil microorganism and important functions such as N cycling. Some of these compounds also represent a source of N to microbial communities through mineralization. For example, the ability of microorganism to use atrazine as a sole N source has been demonstrated (Mandelbaum *et al.*, 1995; Struthers *et al.*, 1998). As bioavailability of pesticides depends on the formulation as well as on diverse crop and soil factors (e.g. percentage crop cover of the soil surface, soil type, structure, pH, N and C contents, pore volume, water-holding capacity) determining sorption, leaching and degradation of the compound, the response of the microbial biomass is expected to be linked to both the soil type and the pesticide used. Moreover, herbicides are typically applied onto bare soil while fungicides and insecticides are used on dense crops and the exposure of the soil is consequently lower (Johnsen *et al.*, 2001).

The effects of pesticides on bacterial groups involved in N transformation have been thoroughly studied using cultivation-dependent methods in the past, for example, *Rhizobium* fixing N in symbiosis with leguminous plants (Aggarwal *et al.*, 1986; Kishinevsky *et al.*, 1988; Mårtensson, 1992; Revellin *et al.*, 1992; Ramos & Ribeiro, 1993; Singh & Wright, 2002), free-living diazotrophs *Azotobacter* and *Azospirillum* (Banerjee & Banerjee, 1987; Jena *et al.*, 1987; Martinez-Toledo *et al.*, 1988) and nitrifying bacteria (Doneche *et al.*, 1983; Banerjee & Banerjee, 1987; Martinez-Toledo *et al.*, 1992a, b). On the contrary, only a few recent studies have used culture-independent approaches to better gain insight into the effects on the structure and function of soil microbial communities (Engelen *et al.*, 1998; Rousseaux *et al.*, 2003; Seghers *et al.*, 2003; Devare *et al.*, 2004; Saeki & Toyota, 2004; Bending *et al.*, 2007). In many cases, pesticides applied at the recommended field rate concentration did not have a significant impact on the structure and function of the soil microbial communities (Saeki & Toyota, 2004; Ratcliff *et al.*, 2006). Seghers *et al.* (2003) demonstrated that the community structure of AOB in bulk soil of a maize monoculture was unaltered by 20 years of atrazine and metolachlor application. Some other studies have indicated more pronounced effects. Thus, Chang *et al.* (2001) observed a severe impact of atrazine on both the abundance and the community structure of AOB. However, in this study, short-term microcosm experiments were performed with high herbicide concentrations (*c.* three orders of magnitude higher than the field rates). There is also increasing evidence that chloropicrin and methyl isothiocyanate can stimulate N_2O production (Spokas & Wang, 2003; Spokas *et al.*, 2005, 2006). For other herbicides like prosulfuron, glyphosate and propanil as well as the fungicides mancozeb and chlorothalonil, decreased N_2O emissions were observed, possibly because the compounds inhibited nitrification and denitrification (Kinney *et al.*, 2005). Cernohlávková *et al.* (2009) confirmed this hypothesis and demonstrated that mancozeb and dinocap can impair nitrification at a field rate in an arable and a grassland soil.

Besides pesticides, antibiotics are also extensively used in agricultural production systems, predominantly in livestock husbandry. As slurry and manure are usually applied as organic fertilizers in agricultural farming, a substantial fraction of the administrated compounds enters the environment (Lamshöft *et al.*, 2007). Unlike pesticides, antibiotics are explicitly designed to affect microorganisms. The impact of, for example, sulfadiazine, a broad-spectrum bacteriostatic agent, has been intensively evaluated due to its frequent use, high excretion rate and persistence in soil (Thiele-Bruhn, 2003; Lamshöft *et al.*, 2007; Schauss *et al.*, 2009a). Similar to pesticides, soil and crop characteristics are major factors influencing the response patterns of the microbial communities toward antibiotics in soil (Heuer & Smalla, 2007; Hammesfahr *et al.*, 2008; Kotzerke *et al.*, 2008; Schauss *et al.*, 2009a; Ollivier *et al.*, 2010). Potential nitrification activity remained unchanged under low sulfadiazine concentration conditions in bulk soil when applied in combination with manure (Kotzerke *et al.*, 2008). This might have been due to a substitution of the highly affected AOB by their archaeal counterparts (Schauss *et al.*, 2009b). Similar observations concerning sulfadiazine effects on the abundance patterns of AOB and AOA were made in the rhizosphere of maize and clover (Ollivier *et al.*, 2010). Also, both functionally redundant groups of nitrite reducers were negatively influenced by antibiotic addition to manure. Hence, not surprisingly, potential denitrification rates decreased in treatments where sulfadiazine was applied (Kotzerke *et al.*, 2008). While nitrite reducers harboring the *nirS* gene increased in abundance after bioavailable sulfadiazine had declined, the abundance of *nirK*-harboring nitrite reducers remained on the level of the nonmanured control treatment (Kleineidam *et al.*, 2010). Clearly, pronounced effects of sulfadiazine on the denitrifying bacteria were also observed in the rhizosphere of maize and clover, where the dominating *nirK*, but also the *nirS* nitrite reducers as well as the *nosZ*-harboring N_2O reducers were significantly impaired (Ollivier *et al.*, 2010). Furthermore, the abundance of *nifH* genes, coding for key enzyme of N fixation, was significantly impacted by sulfadiazine in the rhizosphere of both plant types, but to a greater extent in the rhizosphere of the legume.

Conclusions and outlook

The research over the last two decades linking N transformation processes in soil to the corresponding functional microbial communities has improved our knowledge significantly about the factors driving the abundance, diversity and activity mainly of microorganisms involved in the inorganic N cycle as well as the dynamics of the corresponding turnover processes and nutrient fluxes. Overall, most studies that addressed questions linked to the consequences of land-use changes or agricultural management included data for nitrifiers, denitrifiers and N-fixing microorganisms, whereas studies in the area of climate change in most cases focused only on consequences for denitrification and N_2O emissions. This reflects well the areas of interest of the various scientific communities involved in the different research areas. However, it must be taken into account that the processes of the N-cycle are closely interlinked and thus influence each other. Thus, even if the focus is on trace gas emissions from soil, knowledge of processes like nitrification and N-fixation is of key importance too. In general, data on the diversity and abundance of N-mineralizing microorganisms are rare in microbial ecology, due to the huge variety of different biochemical pathways, which are so far mostly unknown. Therefore, not surprisingly, in most studies that are of relevance for consequences of global change on N-transformation, this functional group of microorganisms has been excluded from analyses. Nevertheless, it is generally accepted that the amount of mineralized nitrogen is one major driver for the inorganic nitrogen cycle mainly in nonfertilized natural soils.

From the recently published data, the following conclusions can be drawn generally: (1) global change-related modifications of environmental factors affect nitrifiers, denitrifiers and N-fixing microorganisms and alter the corresponding processes. (2) The abundance of the autotrophic ammonia oxidizers and nitrite oxidizers in soil is negatively correlated with additional C input by plants as a result of land-use changes towards agricultural land or a more intensive agriculture as well as enhanced CO_2 concentrations in the atmosphere. This results in soils, where no inorganic fertilizer has been applied, in reduced nitrate concentrations and consequently, despite the presence of easily degradable carbon sources, in reduced denitrification activity under anoxic conditions. Although N-fixing microorganisms benefit from the additional carbon input, their activity is only increased under low ammonia concentrations in soil, for example, conditions where most of the ammonia is taken up by the plant or by soil microorganisms for biomass production. Overall, plants might benefit from this scenario due to reduced competition for ammonium with ammonia-oxidizing microorganisms in soil. Furthermore, such conditions may reduce the amount of leached nitrate as well as emissions of N_2O. (3) By contrast, ammonia oxidizers might benefit from the application of xenobiotics as AOA in particular seems to tolerate a number of compounds that, like antibiotics, are toxic for other prokaryotes (Schauss *et al.*, 2009a, b). This may result in increased nitrification rates if enough ammonia is available and consequently in the formation of nitrate. As denitrifiers might be reduced in their activity under the given scenario, nitrate could leach to the ground water, if it is not taken up by the plants. (4) Water conditions and the oxygen content in soil highly influence nitrifiers and denitrifiers. Under anoxic conditions, however, the activity of denitrifiers again depends on the amount of available nitrate and, therefore, either on fertilization regimes or the activity of nitrifiers in non-water-logged habitats in soil.

As stated in the introduction, 'global change' encompasses *interlinked* activities of the different scenarios described above. Because each scenario results in a different response pattern of the investigated microbial communities, a prediction of what happens if two or more scenarios are mixed is almost impossible. For example, whether the addition of xenobiotics and increased carbon inputs by increased atmospheric CO_2 concentrations will lead to higher or lower concentrations of nitrate in soil cannot be predicted from currently available data. However, these types of predictions are needed to transform scientific results into concrete recommendations for practice. Another important aspect of research linked to global change is to understand the long-term consequences of changes in the environment for microbial life in soil. As yet, most studies in the past have concentrated on short-term effects using sometimes highly unrealistic predictions of future conditions. Therefore, in many cases, results represent data more relevant for disturbance ecology than for global change research. As described above, this is true for many experimental setups in the frame of climate change. Finally, the different scales of relevance must be taken into account. Microorganisms act on the μm^2 scale; however, the scales that need to be addressed in terms of political recommendations are at regional or even at a global scale. And conceptual approaches to overcome the scale problem are far from being 'on the market'. This holds true for 'upscaling' from 1 g of soil to the ha or km^2 scale, but also for 'downscaling' 1 g of soil to microsites of μm^2, where microbial life occurs. In this respect, research addressing questions about the relevant scale that must be considered for different scenarios of global change is currently absent.

Authors' contribution

J.O. and S.T. contributed equally to this work.

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