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Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle

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Abstract

The nitrogen (N) cycle contains two different processes of dissimilatory nitrate (NO_3^-) reduction, denitrification and dissimilatory NO_3^- reduction to ammonium (DNRA). While there is general agreement that the denitrification process takes place in many soils, the occurrence and importance of DNRA is generally not considered. Two approaches have been used to investigate DNRA in soil, (1) microbiological techniques to identify soil microorganisms capable of DNRA and (2) ^{15}N tracing to elucidate the occurrence of DNRA and to quantify gross DNRA rates. There is evidence that many soil bacteria and fungi have the ability to perform DNRA. Redox status and C/NO_3^- ratio have been identified as the most important factors regulating DNRA in soil. ^{15}N tracing studies have shown that gross DNRA rates can be a significant or even a dominant NO_3^- consumption process in some ecosystems. Moreover, a link between heterotrophic nitrification and DNRA provides an alternative pathway of ammonium (NH_4^+) production to mineralisation. Numerical ^{15}N tracing models can be particularly useful when investigating DNRA in the context of other N cycling processes. With this review we summarise the importance and current knowledge of this often overlooked NO_3^- consumption process within the terrestrial N cycle. We strongly encourage considering DNRA as a relevant soil N process in future N cycling investigations.

1 Introduction

Our understanding of the nitrogen (N) cycle has increased in recent years due to the discovery of new processes and that various groups of microorganisms are involved in N transformations, e.g. archaeal ammonia (NH_3) oxidation and codenitrification (Hayatsu et al., 2008; Francis et al., 2007). Among the many processes that constitute the N cycle are two processes of dissimilatory nitrate (NO_3^-) reduction that occur under similar conditions of low oxygen concentrations (Tiedje et al., 1982): denitrification, which is the reduction of NO_3^- to gaseous N compounds (NO, N_2O and N_2), and dissimilatory

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NO_3^- reduction to ammonium (NH_4^+) (DNRA), which is also termed fermentative NO_3^- reduction, NO_3^- ammonification or fermentative ammonification. In both processes nitrite (NO_2^-) is an intermediate product (Philippot and Højberg, 1999). Hence, our discussion on DNRA is in most points equally valid for dissimilatory NO_3^- and NO_2^- reduction.

As early as 1938, Woods showed that DNRA occurs in common soil bacteria like *Clostridium welchii* and concluded that DNRA "must be seriously considered in assessing the importance of the oxidation of NH_3 to NO_3 by other micro-organisms in the general circulation of N in nature" (Woods, 1938). This view was supported later by Stanford et al. (1975) who used ^{15}N tracing techniques. They stated that "results seriously challenge the prevalent view that denitrification accounts for essentially all NO_3^- dissimilation in anaerobic soils". However, most investigations still consider denitrification as the only dissimilatory NO_3^- reduction process in soil (Cole, 1990). The principal importance of DNRA is that NO_3^- is transferred into another mineral N form which is less mobile and thus, in contrast to denitrification, may conserve N in the ecosystem (Buresh and Patrick, 1978; Tiedje, 1988). Therefore, DNRA has been called a "short circuit in the biological N cycle" (Cole and Brown, 1980), as the direct transfer of NO_3^- and NO_2^- to NH_4^+ bypasses denitrification and N_2 fixation. Nitrate and NO_2^- reduction during DNRA are catalysed by two different sets of enzymes of which the first is respiratory while the second is fermentative (Moreno-Vivián and Ferguson, 1998; Simon, 2002; Mohan et al., 2004). Since both mechanisms are dissimilatory (Moreno-Vivián and Ferguson, 1998) and can be expected to have similar ecological relevance for N retention, they will be considered together in this review.

Based on a comparison of the potential free energy of total denitrification ($NO_3^- \rightarrow N_2$, –2669 kJ mol^{-1} glucose) and DNRA (–1796 kJ mol^{-1} glucose; Gottschalk, 1986), denitrification should be favoured over DNRA. However, under NO_3^- limiting and strongly reducing conditions, a shortage of electron acceptors is most likely limiting microbial growth. Under these conditions DNRA has the advantage over denitrification since more electrons can be transferred per mole NO_3^- (Tiedje et al., 1982). Additionally

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the potential free energy calculated per NO_3^- is higher for DNRA than denitrification (Tiedje et al., 1982; Strohm et al., 2007). By comparing the growth yield of denitrifier and DNRA bacteria in pure culture, Strohm et al. (2007) showed that the actual energy yield of denitrification was much lower than what was expected from the free energy and even lower than the actual energy yield of DNRA, which was discussed as a consequence of inefficient energy conservation by denitrifiers. Consequently, during DNRA twice as much cell mass was synthesised per mol of NO_3^- compared to denitrification (Strohm et al., 2007). These findings are supporting the hypothesis that the ratio of electron donor to acceptor (i.e. available C to NO_3^-) is an important factor in the partitioning of NO_3^- reduction between denitrification and DNRA (Tiedje et al., 1982). DNRA is favoured under higher C/NO_3^- ratios when the electron acceptor (NO_3^-) becomes limiting (Tiedje et al., 1982).

While the importance of DNRA in marine ecosystems (Burgin and Hamilton, 2007), the responsible enzymes and bioenergetics (Philippot and Højberg, 1999; Simon, 2002; Takaya, 2002) as well as molecular techniques to track DNRA microorganisms (Philippot, 2005) were recently reviewed no such current review is available for DNRA in soils. In recent years N cycling studies have increasingly investigated DNRA in various ecosystems. Thus we think it is timely to revisit this often forgotten process, summarise the current knowledge of DNRA in terrestrial ecosystems and to explore its importance for soil N cycling. We will discuss how various environmental factors influence DNRA in soil and approaches to investigate the importance of DNRA in soil. Two approaches have been used: first, microbiological techniques have been applied to identify soil microorganisms capable of performing DNRA and to assess their abundance, in particular in comparison with denitrifying microbes; second, ^{15}N has been used as a tracer to qualitatively investigate NH_4^+ production from added $^{15}NO_3^-$ in order to elucidate if DNRA occurs in soil. Moreover, ^{15}N tracing techniques also allow the quantification of gross rates for DNRA, which will be highlighted in this review.

2 Environmental conditions for DNRA

The soil oxidation state is a principal factor that influences the importance of DNRA compared to denitrification (Matheson et al., 2002; Brunel et al., 1992) with DNRA by bacteria and fungi occurring under more reducing (anoxic) conditions (Takaya, 2002; Yin et al., 2002; Page et al., 2003). Other studies showed that DNRA is less sensitive to variable redox conditions (Pett-Ridge et al., 2006) and less sensitive to O_2 than denitrification (Fazzolari et al., 1998). In the later study soil aggregates were incubated under various O_2 levels with the same NO_3^- concentrations combined with different glucose C additions. The authors showed that the effect of variable O_2 on DNRA was dependent on the C/NO_3^- ratio and concluded that C rather than O_2 was the key factor regulating NO_3^- partitioning between denitrification and DNRA (Fazzolari et al., 1998). This study as well as the one by Smith (1982) confirmed the importance of the C/NO_3^- ratio on partitioning NO_3^- reduction between DNRA and denitrification (Tiedje et al., 1982). Yin et al. (1998) showed that significant DNRA occurred only at a C/NO_3^- ratio above 12. However, Matheson et al. (2002) argued that the effect of C/NO_3^- ratio on DNRA may be an artefact of experimental approaches. Experiments investigating DNRA under different C/NO_3^- ratios usually artificially alter either the organic C or NO_3^- content that result in stimulation of microbial activity and hence O_2 consumption or oxidising of the soil itself by NO_3^- addition (Matheson et al., 2002). Therefore, Matheson et al. (2002) concluded that experimental evidence of the effect of C/NO_3^- ratio are most likely due to altered soil oxidation state, which is hence the key partitioning factor. However, in a tropical forest the natural difference in the C/NO_3^- ratio explained 44% of the variability of gross DNRA rates determined by an in-situ ^{15}N tracing experiment (Silver et al., 2005). In contrast, in a lab incubation of intact soil cores from another tropical forest no correlation between DNRA and C/NO_3^- was detected (Sotta et al., 2008). These contrasting results could be due to the occurrence of DNRA in anaerobic micro-sites and that the bulk soil C/NO_3^- ratio may not be a representative indicator for the ratio at the site of activity.

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Tiedje et al. (1988) pointed out that it is the availability of a suitable organic C source, supporting respiration or fermentation, that regulates the population of DNRA bacteria. In several studies it was shown that the addition of glucose, a carbohydrate that supports respiration as well as fermentation, stimulated DNRA (Buresh and Patrick, 1978; Smith and Zimmerman, 1981; Yin et al., 2002; Caskey and Tiedje, 1979; Fazzolari Correa and Germon, 1991; Yin et al., 1998). In a ^{15}N labelling study with soil slurries Morley and Baggs (2010) reported that DNRA appeared to be stimulated more by carbohydrates (glucose and mannitol) than amino acids and butyrate, but Yin et al. (1998) reported that the carbohydrates glycerol and succinate do not support DNRA. Moreover, in two anaerobic soils, addition of glucose did not influence DNRA (Chen et al., 1995). Chen et al. (1995) identified several possible explanations, including the high native soil C content compared to the amount of added glucose, unfavourable redox conditions for DNRA or due to soil rewetting. DeCatanzaro et al. (1987) also found no effect of glucose addition on DNRA, but revealed that DNRA was stimulated by alfalfa addition. This was apparently an effect of sulphur in alfalfa, which was released by decomposition as -SH group of organic matter and served as reducing agent. Under anaerobic conditions, sulphide stimulates DNRA, by serving as an electron donor, and depresses denitrification, by repressing NO and N_2O reductase (Myers, 1972; Brunet and Garcia-Gill, 1996). In this line DeCatanzaro et al. (1987) found in the above mentioned study a stimulation of DNRA when simultaneously adding glucose and sulphide, which contrasted the finding from only glucose addition.

Other added C sources like straw, glycerol, methanol and succinate were found to not promote DNRA (Buresh and Patrick, 1978; deCatanzaro et al., 1987; Yin et al., 1998). The reasons for this are not fully understood and deserve further investigations. Buresh and Patrick (1978) as well as Yin et al. (1998) attributed this finding to the fact that the mentioned C sources are poor substrates for fermentation. As DNRA was thought to be a solely fermentative process (Cole and Brown, 1980) these substrates hence also did not promote DNRA. However, as two distinct pathways of DNRA exists, one fermentative and one respiratory (Moreno-Vivián and Ferguson, 1998; Simon, 2002;

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Mohan et al., 2004), this can not be the sole explanation. For the respiratory DNRA Simon (2002) listed formate, H_2 and sulphide as substrates. Therefore, it may be likely that the above mentioned C sources do neither favour the fermentative nor the respiratory DNRA pathway. This may also explain while in some cases addition of glucose does not support DNRA and some of the above mentioned contradictions in the response to C/NO_3^-, as this may depend on if respiratory or fermentative DNRA bacteria are present. This is support by the above discussed findings by DeCatanzaro et al. (1987) in respect of sulphide.

Several studies investigated the effect of pH on DNRA, though findings were partly contradictory. Higher DNRA was associated with alkaline conditions (Nõmmik, 1956; Stevens et al., 1998; Fazzolari Correa and Germon, 1991; Gamble et al., 1977) and Woods (1938) reported a pH optimum of 6.5 for NO_2^- reduction and of 7.5 for NO_3^- reduction. In contrast other studies found a negative relationship between DNRA and soil pH (Davidson and Ståhl, 2000; Waring and Gilliam, 1983). For denitrification many studies found an effect of pH, however it appeared that this effect may be indirect due to changes in the availability of organic C (Šimek and Cooper, 2002). Under acidic conditions the breakdown of organic matter is slowed that in turn reduces the availability of organic C for microorganisms and, hence, denitrification. It is unknown if this is also the case for DNRA, but Waring and Gilliam (1983) reported that DNRA increased at lower pH (<4) in poorly drained soils, which was linked to the soluble C content. Therefore, contrasting findings of the pH effect on DNRA may partly be related to soil C availability and, hence, be of indirect nature.

Taken together, the oxidation status and the C/NO_3^- ratio were reported to be the most important factors regulating the importance of DNRA in soil, while the effect of pH was not consistent. Moreover, other investigations found a correlation between DNRA and SOM, moisture or soil N (Gamble et al., 1977; Davidson and Ståhl, 2000). However, there are not enough data available in the literature to make a comprehensive analysis on the importance of the various factors. Hence, future studies are needed to systematically investigate the main controlling factors of DNRA in soil.

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2.1 Effects of plants on DNRA in soils

The presence of roots alters the activity and abundance of dissimilatory NO_3^- reducers in soils, as a consequence of altered substrate and oxygen availability (Philippot et al., 2009). It is well established that denitrification is generally stimulated by roots (Klemedtsson et al., 1987; Woldendorp, 1963). However, so far no study has investigated the direct effect of plants on DNRA in upland soils, but some information is available for wetland/freshwater plants. Even though these ecosystems are not the focus of this review, the findings, which are not conclusive, are summarised briefly. In the presence of reed sweetgrass (*Glyceria maxima*) DNRA bacteria (53%) dominated the NO_3^- reducer community in a pot experiment (Nijburg and Laanbroek, 1997a), while in unplanted soil denitrifiers dominated (71%). In contrast, the presence of reed (*Thypha angustifolia*) had little effect on the functional groups of NO_3^- reducers, with DNRA bacteria accounting for 12 and 19% in the bulk and rhizospheric sediment from a freshwater lake, respectively (Brunel et al., 1992). A higher contribution of DNRA to the recovery of added $^{15}NO_3^-$ was found in soil cores containing reed roots compared to root free cores (Nijburg and Laanbroek, 1997b). In contrast a $^{15}NO_3^-$ labelling microcosm study found that DNRA accounted for 49% of NO_3^- consumption in unplanted soil, while in the presence of plants DNRA accounted for less than 1% (Matheson et al., 2002). At the same time denitrification was higher in the planted soil. This later study agrees with findings from a riparian zone that during the growing season denitrification and during dormancy, when plant activity was low, DNRA predominated (Dhondt et al., 2003).

Effective soil N retention is achieved by a tight coupling of DNRA with plant (and microbial) NH_4^+ uptake as was observed in tropical upland soils (Templer et al., 2008). This highlights the need to better understand plant interactions with DNRA and N retention in upland soils by conducting parallel studies with planted and unplanted soils. Apart from assimilation, N retention may also occur due to adsorption of NH_4^+, produced via DNRA, on clay minerals or organic matter, and is governed by the cation exchange capacity.

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3 Production of N_2O during DNRA

DNRA is generally seen as a process that conserves N in the ecosystem. However, many microorganisms conducting DNRA also produce N_2O (Cole, 1988). Kaspar (1982) suggested that N_2O production by DNRA microorganisms is a detoxification mechanism, in order to avoid high concentrations of NO_2^-. In a batch culture a soil *Citrobacter* sp. produced N_2O and NH_4^+ by enzymatically reducing NO_2^- (Smith, 1982). The use of $^{13}NO_3^-$ labelling proved that several microorganisms were able to simultaneously produce NH_4^+ and N_2O via dissimilatory pathways, whereby NH_4^+ accounted typically for $> 90\%$ of the total product (Bleakley and Tiedje, 1982). This finding is in line with an anaerobic batch incubation study where all DNRA isolates from three different soils showed N_2O production, which accounted for 5–10% of added NO_3^- (Smith and Zimmerman, 1981). However, as stated by Cole (1988), the N_2O production rate by DNRA microorganisms is typically in the range of 1% of the NO_2^- or NO_3^- reduction. Based on a $^{15}NO_3^-$ labelling study Stevens et al. (1998) concluded that DNRA became a more important process for N_2O production with increasing pH, possibly as a mechanism to reduce harmful NO_2^- that tended to accumulate under high pH. However, as both DNRA and denitrification use the same substrates (NO_3^- and NO_2^-) the contribution of these two processes to total N_2O production can not be investigated based on $^{15}NO_3^-$ labelling alone. Thus the identification of the responsible microorganisms is required (Stevens et al., 1998). To achieve this quantifying enzyme activity rather than investigating microbial species or functional genes is needed, as discussed for linking denitrifiers density to functioning by Philippot and Hallin (2005).

4 Soil microorganisms involved in DNRA

The aim of this section is not to give a comprehensive overview of microorganisms known to be capable of DNRA but to summarise reported soil microorganisms and

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their abundance, particularly in comparison with denitrifiers. The capability for NO_3^- respiration and for DNRA is widely spread among bacteria (Philippot, 2005; Simon, 2002). Tiedje (1988) listed several genera of soil DNRA bacteria, which are either obligate anaerobes (*Clostridium*), facultative anaerobes (*Citrobacter, Enterobacter, Erwinia, Escherichia, Klebsiella*) or aerobes (*Bacillus, Pseudomonas*). In addition a soil *Arthrobacter* strain, an abundant soil genus worldwide which is regarded as an obligate aerobe, showed DNRA when incubated anaerobically (Eschbach et al., 2003) as was also shown for eight *Nitrobacter* strains, that were regarded as obligate aerobe NO_2^- oxidiser (Freitag et al., 1987). Moreover, the capability for DNRA is widely distributed among common soil fungi, mostly belonging to the ascomycota (Zhou et al., 2002). Many bacteria capable of DNRA are found in the Enterobacteriacea, which is the only group of procaryotes with no known denitrifier (Zumft, 1997). Mohan and Cole (2007) pointed out that there is no known bacterium for which it is well established to be capable of both, denitrification and DNRA. Recently Behrendt et al. (2010) provided evidence by growth tests that two new described *Paenibacillus* species, including one fen soil isolate, showed a versatile metabolism and were capable of heterotrophic nitrification, DNRA and denitrification. However, it appears that a final confirmation of this finding is pending. On the other hand, Zhou et al. (2002) showed that denitrification and DNRA are alternatively expressed in a common soil fungus (*Fusarium oxysporum*) depending on oxygen status and available C source (Zhou et al., 2002). These authors classified the metabolism of this fungus depending on O_2 status as: DNRA under anoxic conditions, denitrification when O_2 supply was limited and aerobic respiration under sufficient O_2 supply.

Microorganisms which reduce NO_3^- via a dissimilatory pathway can be classified either as (a) denitrifiers, producing gaseous N compounds, (b) NO_2^- accumulators which reduce NO_3^- only to NO_2^-, or (c) DNRA microorganisms reducing NO_3^- or NO_2^- to NH_4^+. Several authors compared the abundance of DNRA and denitrifying bacteria in soils. This, however, does not provide information on the activity of these bacterial groups in soil, which would require alternative approaches, e.g. ^{15}N labelling as discussed below

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(Sect. 5). Evidence for a direct competition between DNRA bacteria and denitrifiers for NO_3^- comes from a soil inoculation study using $^{15}NO_3^-$ as a tracer (see Sect. 5) (Fazzolari et al., 1990). When a non-sterile soil was inoculated with the DNRA bacterium *Enterobacter amnigenus* the production of $^{15}NH_4^+$ from $^{15}NO_3^-$ increased compared to non-inoculated control soil while at the same time N_2O production decreased. In contrast, inoculation with the denitrifier *Agrobacterium radiobacter* resulted in faster N_2O production while no $^{15}NH_4^+$ was produced. Simultaneously inoculation with both bacteria resulted in intermediate results (Fazzolari et al., 1990).

Studies, investigating microorganisms with a dissimilatory NO_3^- reduction pathway, often found that non-denitrifying NO_3^- reducers were most abundant. In 19 soils, the number of NO_2^- accumulators outnumbered in all cases the number of denitrifiers, with an average ratio of 4:1 (Gamble et al., 1977). This is consistent with the ratio of 4:1–3:1 for DNRA bacteria to denitrifiers in soil reported by Tiedje et al. (1982) and agrees with Bengtsson and Bergwall (2000) who reported a higher number of DNRA bacteria than denitrifier for a spruce forest soil. Moreover, Smith and Zimmerman (1981) found that non-denitrifying bacteria dominated NO_3^- reducers, but most were NO_2^- accumulators. However, the majority of NO_2^- accumulators were capable of DNRA when NO_3^- was limited (Smith and Zimmerman, 1981), which was also found in a *Klebsiella* sp. (Dunn et al., 1979). In contrast Brunel et al. (1992) found that, after addition of glycerol, only few strains of NO_2^- accumulator were capable of DNRA but more were able to produce N_2O. In this context it is interesting to notice that the growth of the DNRA bacterium *Enterobacter amnigenus* was only related to NO_3^- reduction to NO_2^- but not to the reduction of NO_2^- to NH_4^+ (Fazzolari et al., 1990). These authors concluded that NO_2^- reduction may serve as an electron sink but not for energy generation. In three paddy soils, the number of DNRA bacteria was only 19–35% of the number of denitrifiers (Yin et al., 1998). However, in two other paddy soils Yin et al. (2002) found that the number of DNRA was higher than denitrifiers when the soil was pre-incubated or when C was added, which again points to the importance of C as discussed in Sect. 2.

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However, all the discussed results are based on culturable microorganisms and, moreover, the activity of a DNRA bacterium was shown to differ between pure culture and soil inoculation (Fazzolari et al., 1990). Investigating the abundance of bacteria by function genes, Kandeler et al. (2009) reported that denitrifiers accounted for less than half of the total NO_3^- reducer community in a forest soil. Molecular approaches for studying the microbial community of NO_3^- reducers in-situ were recently reviewed by Philippot (2005) and are therefore not repeated here. The review by Philippot (2005) highlighted the importance of functional genes, of culture independent approaches and of quantitative information when investigating denitrifier and NO_3^- reducer communities. Moreover, to link microbial diversity with functional activity Philippot and Hallin (2005) pointed out the need for investigating enzyme activities rather than functional genes (DNA as well as mRNA). This could potentially also provide a strong tool for investigating and comparing the DNRA and denitrification activity in soil.

5 Investigating DNRA by ^{15}N tracing techniques

^{15}N tracing techniques are commonly used to investigate the fate of N in terrestrial ecosystems (Hart and Myrold, 1996). These techniques are also used to quantify gross transformation rates (see below). To confirm the occurrence of DNRA in soil, various researchers applied $^{15}NO_3^-$ and measured the ^{15}N enrichment of NH_4^+ after incubation. Commonly, these studies assumed that NO_3^- immobilisation was negligible due to high NH_4^+ concentrations. If NO_3^- immobilisation occurred at significant rates, ^{15}N enrichment of NH_4^+ may also be the result of immobilisation (i.e. assimilatory NO_3^- reduction) and subsequent remineralisation. However, there seems to be no study that has systematically investigated if this assumption, negligible NO_3^- immobilisation, holds true.

More than 50 yr ago Nõmmik (1956) showed that a small amount of added $^{15}NO_3^-$ was converted to NH_4^+, but only under strictly anaerobic conditions. Therefore, it was

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concluded that DNRA is "extremely insignificant" under the prevailing conditions in arable soil (Nõmmik, 1956). This was also true for six tropical soils from the Philippines, where only a small fraction (< 2%) of added $^{15}NO_3^-$ was recovered as NH_4^+ (MacRae et al., 1968). After incubating ten agricultural soils with varying texture and properties, Fazzolari Correa and Germon (1991) showed that 10–38% of added $^{15}NO_3^-$ was reduced to NH_4^+ via DNRA when a labile C source was added, which is in the same range as found by Stanford et al. (1975) and Wan et al. (2009). In these studies only very small amounts of $^{15}NO_3^-$ were recovered as $^{15}NH_4^+$ without addition of labile C. However, Fazzolari Correa and Germon (1991) pointed out that all the conditions required for DNRA can be present in agricultural soils. Furthermore, several studies using anaerobic soil incubations attributed $^{15}NO_3^-$ recovery as $^{15}NH_4^+$ to DNRA (Buresh and Patrick, 1978; Ambus et al., 1992; Chen et al., 1995; Dhondt et al., 2003; Yin et al., 1998).

In a soil core experiment with soil from a riparian fen, DNRA was only detected below a depth of 5 cm (Ambus et al., 1992). However, when the same soil was incubated as slurry, DNRA did not differ between three soil layers (0–5, 5–10 and 10–20 cm), but the ratio of DNRA to total NO_3^- reduction increased with depth (Ambus et al., 1992). This finding points to the effect that experimental design (e.g. soil slurry vs. core incubation) can have on experimental results, which impedes the comparison of results from different studies. Another riparian buffer zone study using slurry incubations showed that DNRA was only significant during the dormant season, which was attributed to low redox potentials and high inputs of labile C during that time (Dhondt et al., 2003). The studies by Ambus et al. (1992) and Dhondt et al. (2003) both point to the occurrence of DNRA under more reduced conditions compared to denitrification. However, some studies indicated that DNRA and denitrification can occur simultaneously in soil (Paul and Beauchamp, 1989; Stevens and Laughlin, 1998; Morley and Baggs, 2010), possibly in different micro-sites that differ in their redox state. Furthermore, in contrast to Ambus et al. (1992) slurry incubations with soil from a riparian zone by Davis et al. (2008) resulted in higher DNRA rates in the surface soil (0–15 cm) compared to

sub-soil (130–155 cm). A similar result was observed in an adjacent cropping system. The different results between the two studies may be related to the different soils depth investigated, due to changing substrate availability and redox conditions with depth.

5.1 Gross transformation rates for DNRA

Higher numbers of DNRA microorganisms compared to denitrifiers may not necessarily reflect a higher DNRA activity in soil. Thus, investigations of gross transformation rates are needed to evaluate the importance of DNRA. This can be achieved by ^{15}N labelling techniques in conjunction with data analysis via analytical or numerical models. However, most ^{15}N labelling studies to date did not consider DNRA, because it is assumed to be a negligible process. Here, we will summarise gross DNRA rates reported from soils and explore if DNRA is indeed negligible or must be considered as a significant important N pathway in soil.

The first reported gross DNRA rate measurement in soils was presented by Ambus et al. (1992) for a riparian fen. Unfortunately, no equation for the calculation of the gross rates was provided. An analytical solution to calculate gross rates for DNRA, based on the increase of the ^{15}N enrichment of the NH_4^+ pool after addition of $^{15}NO_3^-$ as a tracer, was developed by Silver et al. (2001) (the actual equations are presented in: Huygens et al., 2008). The derived analytical equations were applied to investigate DNRA in various ecosystems, mostly tropical forest soils (Table 1). Several studies showed that DNRA was a significant and sometimes dominant fate of NO_3^- in terrestrial ecosystems (Table 1). Some studies compared DNRA and denitrification rates. In a tropical forest soil DNRA was threefold higher than denitrification (Silver et al., 2001) and in a spruce forest the gross rate of DNRA was three orders of magnitude higher than gross denitrification (Bengtsson and Bergwall, 2000). This was also found for freshly sampled soil from another tropical forest (Pett-Ridge et al., 2006). However, when this soil was pre-incubated for 3–6 weeks under different redox regimes denitrification exceeded DNRA in all cases. Furthermore, Pett-Ridge et al. (2006) found that DNRA was unexpectedly higher in aerobic soils than in anoxic soils and soils with fluctuating redox conditions.

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They explained this observation by higher NO_3^- concentrations in aerobic soil and the possibility of the occurrence of DNRA in anaerobic soil aggregates. Previously, Silver et al. (2001) showed that the rate of DNRA can be limited by the availability of NO_3^- that is caused by a small pool size in conjunction with high turnover. The DNRA rate constant, calculated as gross rate divided by NO_3^- concentration, was in the Pett-Ridge et al. (2006) study, however, highest in anoxic soils. Comparing the rate constant of denitrification and DNRA revealed that DNRA bacteria are more competitive for NO_3^- under fluctuating redox conditions (Pett-Ridge et al., 2006). Thus, it appears that under certain environmental conditions DNRA bacteria are able to compete successfully with denitrifying bacteria for NO_3^-, which supports the theoretical advantage of DNRA under low NO_3^- concentrations (Tiedje et al., 1982; see Sect. 2).

Unfortunately analytical solutions for quantifying gross transformation rates, and particularly DNRA, introduce inconsistencies. These inconsistencies can occur when the assumption that no ^{15}N is recycled into the labelled pool does not apply, when inappropriate kinetic settings for N transformations are used (Rütting and Müller, 2007) or if NO_3^- consumption and DNRA are calculated separately. Using an analytical model, Templer et al. (2008) found a higher gross rate for DNRA compared to total NO_3^- consumption in one out of three tropical forest soils, which could have been due to inconsistencies. To overcome the problems associated with analytical solutions we recommend using numerical data analysis via so called ^{15}N tracing models (Rütting and Müller, 2007; Rütting et al., 2011), which enables a simultaneous analysis of all NO_3^- consumption pathways in a coherent model framework. This recommendation is in line with Silver at al. (2001) who stated that "numerical modeling may provide an alternative approach to explore the role of DNRA under a variety of scenarios". The only ^{15}N tracing model that included DNRA was presented by Müller at al. (2004, 2007). An alternative approach to numerical tracing models was presented by Tietema and van Dam (1996), who combined ^{15}N experiments with a simulation model. In this model DNRA was simulated as a function of microbial biomass, but was independent of substrate concentrations.

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Application of the ^{15}N tracing model developed by Müller at al. (2004, 2007) showed that DNRA is likely to occur in numerous ecosystems and was sometimes the dominant NO_3^- consumption process (Table 1). However, other investigations did not find evidence for DNRA (Cookson et al., 2006; Laughlin et al., 2008; Inselsbacher et al., 2010), confirming that DNRA may be important only in some, but not all ecosystems (Stanford et al., 1975). Such observations, however, may also be related to the experimental conditions. One additional advantage by using numerical ^{15}N tracing models is that correlations between N transformations can be investigated (Müller et al., 2007). This enabled the detection of functional linkage between DNRA and the organic pathway of heterotrophic nitrification (oxidation of organic N) in a *Nothofagus* forest on an Andisol in Southern Chile (Rütting et al., 2008). The authors considered this link to be an adaptation of the microbial community with the result that N losses were minimised. Recently two new bacterial species were described that performed simultaneously DNRA and heterotrophic nitrification (Behrendt et al., 2010), supporting the above proposed functional linkages. Such a functional link, if proved to be a general pattern in soil, could provide an alternative pathway of NH_4^+ production from soil organic matter to the direct mineralisation (Fig. 1a). In the *Nothofagus* forest DNRA accounted for more than 90% of total NO_3^- consumption (Huygens et al., 2007; Rütting et al., 2008). However, the transfer of ^{15}N from NO_3^- to NH_4^+ could in fact be due to three different pathways (Fig. 1b): (1) DNRA, (2) plant N efflux and (3) remineralisation by microorganisms (Burger and Jackson, 2004). Using data from a microcosm ^{15}N study and simulation models Burger and Jackson (2004) provided evidence that each of the three pathways was on its own able to explain the observed ^{15}N dynamics. Numerical ^{15}N tracing models (e.g. Müller et al., 2007) have the potential to investigate the most likely pathway of NO_3^- reduction to NH_4^+ when the ^{15}N enrichment of roots, soil organic N and microbial biomass are measured in addition to the mineral N pools (Fig. 1b). The alternative pathways should be tested to identify via a likelihood analysis whether DNRA or alternative pathways most likely occurred. In the above mentioned *Nothofagus* study these alternative pathways (plant N efflux and remineralisation) could be

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ruled out as no roots were present in the laboratory incubation and the ^{15}N enrichment in five organic N fractions was too low to explain the $^{15}NH_4^+$ enrichment by remineralisation (Rütting et al., 2008; Huygens et al., 2007). More detailed studies are needed to investigate the importance of the alterative pathways proposed by Burger and Jackson (2004), i.e. DNRA, plant N efflux and remineralisation by microorganisms, by combining ^{15}N labelling studies with numerical data analysis.

Recently, two studies showed that forest type influences the importance of DNRA (Zhang et al., 2011; Staelens et al., 2011). In both studies soils forested with either broad-leaf or coniferous tree species were compared and higher DNRA rates were observed in soil underneath broad-leaf species (Table 1). These differences may be related to the fact that broad-leaves contain usually a higher amount of labile C compared to coniferous needles, which may stimulate DNRA (see Sect. 2). It was also shown that the soil type had a significant effect on DNRA, with higher rates in clay compared to sandy soil (Sotta et al., 2008), but no explanation was provided.

6 Effect of Global change on DNRA

The functional importance of DNRA in soil is its capacity to increase N retention, as NO_3^- is transformed to NH_4^+. Ammonium is available for plant and microbial uptake, but is less prone to losses via leaching or as gaseous compounds (Buresh and Patrick, 1978; Tiedje, 1988; Silver et al., 2001; Huygens et al., 2007). Current climate change scenarios suggest that many ecosystems may become more N limited in the future. This is mainly due to increased atmospheric CO_2 concentration, which can lead to a higher plant N demand (Hungate et al., 2003; Luo et al., 2004). The possible N limitation might be partially alleviated by increasing N deposition (Johnson, 2006). However, Hungate et al. (2003) showed that the expected increase in N deposition will not cover the additional N demand under elevated CO_2, indicating that N retention processes such as DNRA may become more important for ecosystem productivity. Tietema and van Dam (1996) investigated the effect of increased N deposition on the N cycle in two

coniferous forest soils. At one site they found higher DNRA under pre-industrial (1–2 kg N ha^{-1} yr^{-1}) compared to increased deposition (31–37 kg N ha^{-1} yr^{-1}), while no effect was found for the second forest. In a forest N fertilisation experiment in Sweden no clear pattern of the relationship between the amount of fertiliser applied (0–180 kg N ha^{-1} yr^{-1}) and $^{15}NO_3^-$ reduction to NH_4^+ was found (Bengtsson and Bergwall, 2000). Kandeler et al. (2009), however, found lower total nitrate reductase activity in soil where N deposition was decreased, although the total number of nitrate reduction genes was not affected. The effect of elevated CO_2 on N cycling rates, including DNRA, was recently investigated in soils from two long-term free air CO_2 enrichment studies on temperate grassland (Müller et al., 2009; Rütting et al., 2010). In both of these studies DNRA was stimulated under elevated CO_2 by 140 and 44%, respectively, most likely due to an increased C input into the soil that stimulated microbial activity and possibly increased anaerobicity.

7 Conclusions

Thirty years ago Cole and Brown (1980) concluded that the significance of DNRA in anaerobic soil was unknown. Now, with the use of ^{15}N labelling techniques and the quantification of gross DNRA rates, the hypothesis that DNRA "may be much more important than presently realized" (Stevens et al., 1998) seems to be confirmed. Gross DNRA rates can be quantified via ^{15}N tracing studies in combination with numerical data analysis and ^{15}N tracing models that consider DNRA as well as all N transformations that interact with each other (Rütting et al., 2011). A particularly powerful tool for future investigations can be the combination of ^{15}N tracing and molecular approaches (Wallenstein and Vilgalys, 2005; Philippot and Hallin, 2005). Summarising the findings of several studies it can be concluded that DNRA can be a significant or even the dominant NO_3^- consumption process in some ecosystems (Table 1) and the importance of DNRA may increase under the ongoing climate change. Previously, it was concluded that the potential for significant DNRA exists in most soils, but that it is only expressed

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under anoxic conditions when C is readily available, possible in anaerobic micro-sites (Smith and Zimmerman, 1981; Caskey and Tiedje, 1979). Yin et al. (1998) showed that a soil C/NO_3^- ratio above 12 seems to be a threshold for significant DNRA activity, but more studies are needed to ascertain if this threshold is a general feature or variable depending on soil properties. For soils, it appears that more work is needed to understand the importance of DNRA in various ecosystems, as was recently also concluded for aquatic systems (Burgin and Hamilton, 2007). Therefore, future investigations on the soil N cycle in different terrestrial ecosystems (forest, agricultural land, grasslands, wetland) should focus not only on "classical" N cycling transformation such as nitrification and mineralisation, but also should include processes such as DNRA, because the occurrence of this process is often an indicator for ecosystem N retention. Thus the N mineralisation paradigm of Schimel and Bennett (2004) should also be adapted to consider DNRA as an alternative NH_4^+ producing process, in particular in conjunction with the postulated link to the organic pathway of heterotrophic nitrification (Fig. 1). An improved understanding of the conditions that govern whether NO_3^- is reduced to gaseous N or NH_4^+ could also provide possible mitigation scenarios for N_2O.

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Table 1. Summary of gross DNRA rates ($\mu g\, N\, g^{-1}$ soil day^{-1}) in terrestrial ecosystems (mean ± standard deviation, if available, or range of values) calculated by analytical (A) or numerical (N) ^{15}N tracing models as well as the portion of DNRA to total gross NO_3^- consumption (% C_{NO_3}).

Ecosystem	Country[a]	Method[b]	Model	DNRA rate	% C_{NO_3}	Ref.
Riparian fen	DNK	Lsc	A	0.36	3.5	1
Riparian zone	USA	Lsl	A	1.3	n.a.	2
Paddy field	JPN	Lh	A	< 1.2	< 10	3
Temperate forest						
Pine/Douglas fir	NLD	Lh	N	0.01–0.25	11.1–31.8	4[c]
Spruce	SWE	Lh	A	0.07	4	5[d]
Nothofagus	CHL	Fi	A	1.00 ± 0.20	28.7	6
Nothofagus	CHL	Lh	N	0.448 ± 0.024	91.2	7
Nothofagus	CHL	Lh	N	0.355 ± 0.016	98.3	8
Oak	BEL	Fi	N	0.012 ± 0.001	1.7	9
Pine	BEL	Fi	N	0.004 ± 0.002	0.4	9
Subtropical forest						
broadleaf	CHN	Lh	N	0.021 ± 0.003	15.6	10
coniferous	CHN	Lh	N	0.015 ± 0.008	2.3	10
Tropical forest						
Montane	PRI	Fi	A	0.6 ± 0.1	75.0	11
Wet	PRI	Lh	A	0.5–1.2	n.a.	11
Humid	PRI	Lsc	A	2.89 ± 0.57	n.a.	12[e]
Plantation	CRI	Fi	A	0.23 ± 0.12	10.6	13
Old-growth	CRI	Fi	A	0.24 ± 0.08	4.6	13
Plantation	CRI	Lh	A	0.33 ± 0.12	n.a.	13
Lowland	BRA	Lsc	A	0.3–0.8	12.1–50.0	14
Humid	PRI	Fi	A	0.03–1.27	2.2–119.8	15[f]
Temperate grassland						
Ryegrass field	USA	Lsl	A	0.2	n.a.	2
Meadow	GER	Lh	N	0.07	13.8	16
Meadow	GER	Lh	N	0.090 ± 0.003	73.0	17
Meadow	GER	Lh	N	0.090 ± 0.003	96.9	18
Meadow	GER	Lh	N	0.27 ± 0.01	28.1	19
Pasture	NZL	Lh	N	0.034 ± 0.002	0.9	20

[a] BEL – Belgium; BRA – Brazil; CHL – Chile; CHN – China; CRI – Costa Rica; DNK – Denmark; GER – Germany; JPN = Japan; NLD – The Netherlands; NZL – New Zealand; PRI – Puerto Rico; SWE – Sweden; USA – United States of America.
[b] Fi = Field incubation; Lh = Lab – homogenised; Lsc = Lab – soil cores; Lsl = Lab – slurry.
[c] DNRA rates were calculated by a simulation model as function of microbial biomass.
[d] Calculated based on data presented in Table 1 in the original publication for the unfertilised stand.
[e] Gross rate of initial redox treatment.
[f] In one out of three forest soils the rate of DNRA was higher then total NO_3^- consumption.
References: 1 Ambus et al. (1992); 2 Davis et al. (2008); 3 Nishio (1994); 4 Tietema and van Dam (1996); 5 Bengtsson and Bergwall (2000); 6 Huygens et al. (2008); 7 Huygens et al. (2007); 8 Rütting et al. (2008); 9 Staelens et al. (2011); 10 Zhang et al. (2011); 11 Silver et al. (2001); 12 Pett-Ridge et al. (2006) ; 13 Silver et al. (2005); 14 Sotta et al. (2008); 15 Templer et al. (2008); 16 Müller et al. (2004); 17 Müller et al. (2007); 18 Rütting and Müller (2008); 19 Müller et al. (2009); 20 Rütting et al. (2010).

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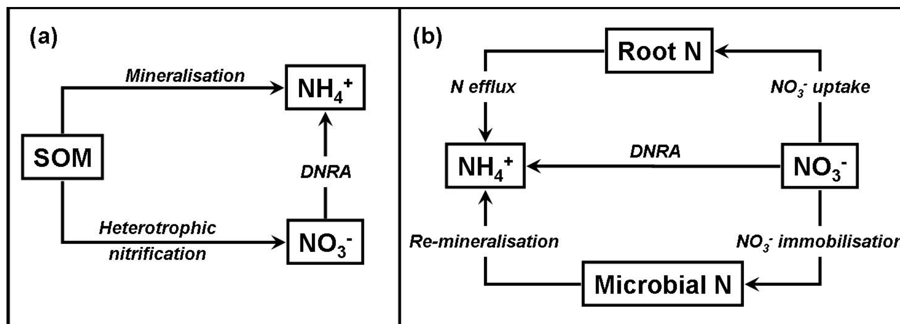

Fig. 1. Schematic overview of the importance of DNRA in soil: **(a)** as an alternative NH_4^+ producing process, when coupled to the organic pathway of heterotrophic nitrification, to direct mineralisation of soil organic nitrogen (SON); **(b)** alternative pathways transferring NO_3^- to NH_4^+ in soil (Burger and Jackson, 2004) that can be evaluated by ^{15}N tracing studies in combination with numerical data analysis if all the shown N pools are measured.

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