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Journal of Agricultural Science (2014), **152**, S34–S44. © Cambridge University Press 2013
doi:10.1017/S0021859613000610

NITROGEN WORKSHOP SPECIAL ISSUE PAPER

Advances in understanding nitrogen flows and transformations: gaps and research pathways

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(*Received 28 February 2013; revised 20 June 2013; accepted 3 July 2013; first published online 19 September 2013*)

SUMMARY

The nitrogen (N) cycle is one of the best studied elemental cycles. However, the N flows and transformations, in particular in aggregated soils, at small scales and in plant–soil systems are not yet fully understood. Analytical and molecular techniques are now available to address N dynamics at small scales. The methodological advances should go hand in hand with the development of suitable mathematical models addressing the small scale and the full complexity of the many interacting effects. The importance of denitrification within the N cycle is highlighted and used as an example of the progress achieved in recent times. Research gaps and possible research pathways are outlined.

INTRODUCTION

The world population is rapidly increasing and is expected to reach *c*. 9 billion in the middle of the 21st century with projected associated effects on all terrestrial ecosystems (Barnosky *et al*. 2012). To feed an increasing world population and enhance agricultural production, a parallel increase in nitrogen (N) consumption has been observed. The increasing amount of N, predominantly originating from industrial N fixation, has contributed to an increase in N availability in terrestrial ecosystems and in particular has enhanced the speed with which N is cycled in the environment, i.e. both the rate at which N has been added to but also lost from the environment has increased (Fig. 1). This increase of available N in the environment is also responsible for enhanced N deposition in all agriculturally dominated landscapes since the mid-19th century and will further increase till the mid-21st century (Galloway *et al*. 2004, 2008). Thus, anthropogenically generated reactive N cascades throughout the global environment (Galloway & Cowling 2002) and the increasing N availability affects the N flows and transformations, in particular the relative dynamics of mineral and organic N that are important indicators of the extent of human interference with ecosystem N dynamics (Schimel & Bennett 2004). Reactive N may be lost from ecosystems as nitrate (NO_3^-), via leaching, or in gaseous N forms such as ammonia, nitric oxide (NO), nitrous oxide (N_2O) or dinitrogen (N_2). These N losses are of significance both economically and environmentally. Despite more than 150 years of N cycling research, starting with studies by well-known scientists such as Liebig (van der Ploeg *et al*. 1999), Bausingault (Aulie 1970), Winogradsky (Ackert 2006) and others, there are still significant questions to be addressed with respect to N transformations and losses from terrestrial ecosystems. In particular, the interactions and impact of plants with the N cycle (Knops *et al*. 2002), the key processes for the N cycle, in particular denitrification (Davidson & Seitzinger 2006) and small-scale variability, and microsite N dynamics and interactions are to a great extent unresolved and not adequately understood. The present paper attempts to provide a brief overview of some current thinking in these areas and also attempts to review and suggest modelling concepts and new analytical approaches that might help to elucidate N flows and pathways in terrestrial ecosystems.

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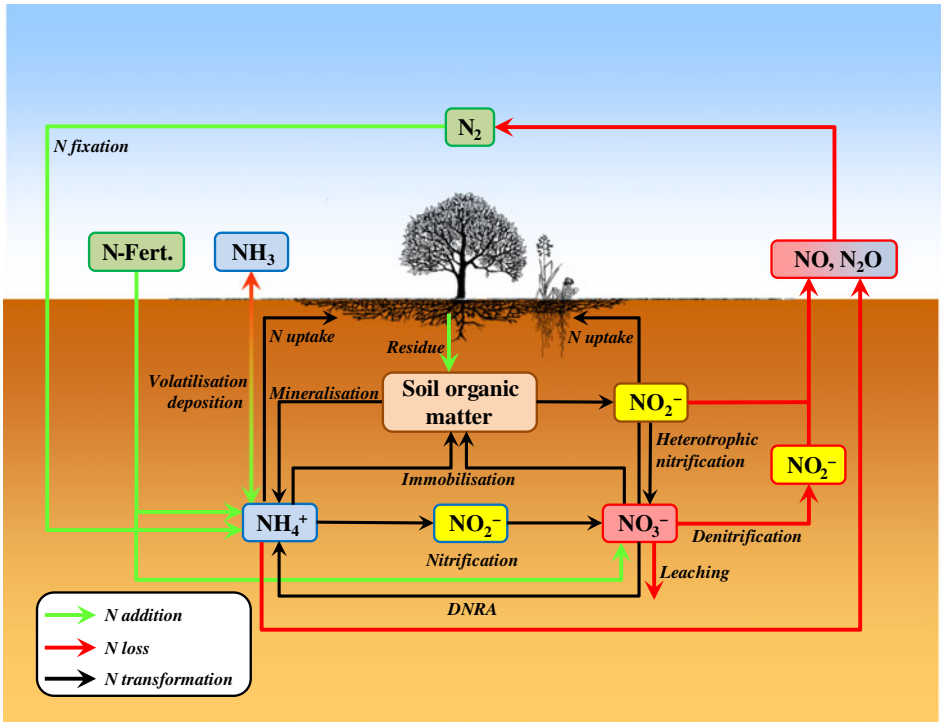


Fig. 1. Terrestrial N cycle with the major N additions (green) and N losses (red) and internal N transformations (black) (colour version available online).

INTERACTIONS OF PLANTS WITH THE N CYCLE

Plant–soil interactions are an area of research attracting increasing interest with respect to N cycling. They are predominantly governed by interactions between the carbon (C) and N cycles. The microbial loop in soil is driven by recent plant C inputs (e.g. rhizodeposition), which influences the microbial activity in soils, whereas ecosystem N availability is controlled by the initial chemical composition and litter N concentrations (Parton *et al.* 2007; Manzoni *et al.* 2008). As the distance from the rhizosphere increases, potential N transformations, such as denitrification, decrease rapidly along a millimetre scale (Beauchamp *et al.* 1989). Thus, soil aggregate–microsite reactions and the influence of roots are likely to cause microscale variations in substrate availability and environmental regulators, prompting a diverse reaction of the microbial community (Barnard *et al.* 2005).

Nitrogen cycling is closely associated with plant productivity and factors affecting it. For instance, conditions that favour plant C assimilation may also enhance rhizodeposition and subsequently alter microbial community composition and function with subsequent effects on N dynamics. Conversely, a higher demand for N by plants, which may occur as a result of environmental change (e.g. favourable growth temperatures), increases competition for N between plants and microbes, potentially affecting microbial community structures and subsequent N transformations in the soil (Zak *et al.* 2003). However, very few studies have examined the links between the N cycle, plant activity and associated changes in microbial form and function that affect N transformations and fluxes. In N-limited systems, the gross N transformation rates and not the sizes of the soil N pools govern the availability of N for plants and microbes (Rastetter *et al.* 1997).

Plants utilize mineral N (e.g. NH_4^+ or NO_3^-) and low molecular organic N compounds (Schimel & Bennett 2004). The latter enter the soil via rhizodeposition (e.g. amino acids) or are made available following microbial mineralization of organic material. Dissolved organic N (DON) uptake is reported to be more significant under conditions of N limitation and low pH (Jones *et al.* 2004). Despite the significance of DON as a loss pathway in agricultural ecosystems (Van Kessel *et al.* 2009), current knowledge of the organic N forms utilized by plants, with respect to soil DON dynamics over time and space, and their rates of production and utilization is still limited

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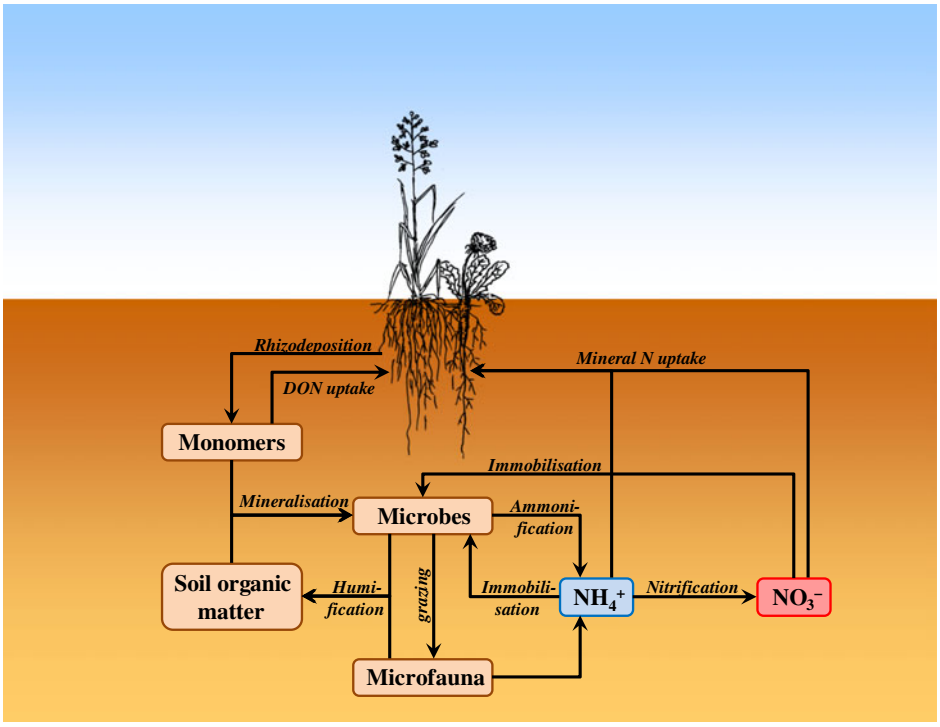


Fig. 2. Major N flows in the rhizosphere (colour version available online).

(Cabrera *et al.* 2005). Similarly, the role DON plays in gaseous N loss pathways is also underresearched. A recent review indicates that nitrosation reactions may be an important loss pathway for gaseous N (Spott *et al.* 2011).

Rhizodeposition stimulates microbial biomass growth and N turnover (Knops *et al.* 2002), which further stimulates the growth of predating organisms such as protozoa and nematodes (Sanderman & Amundson 2003; Osler & Sommerkorn 2007) (Fig. 2). Thus, soil organic matter (SOM) is mineralized/decomposed and may even be accelerated via priming processes (Stockmann *et al.* 2013). So far a quantitative evaluation of the various simultaneously occurring N transformations in plant–soil systems (Fig. 2) that lead to mineralization and immobilization of N in the rhizosphere is lacking. Can plants actively control exudation to create conditions in the rhizosphere to maximize the availability of N? Do plants actively produce compounds that deter non-beneficial organisms/pathogens directly or indirectly via relationships along trophic levels that in turn affect N cycling? Rhizodeposition of C compounds can directly influence N cycling, for instance enhancing N_2O emissions in the presence of an N substrate (Uchida *et al.* 2011). However, relatively little is known about the forms and rates of C released. Research between cell densities and exoenzyme production in the rhizosphere (quorum sensing) are likely to contribute to a quantitative understanding of N dynamics in the rhizosphere. DeAngelis *et al.* (2008) showed that the activity of extracellular enzymes and N mineralization are closely associated and the production of exoenzymes is likely to be controlled by quorum sensing.

N LOSS FROM ECOSYSTEMS – THE IMPORTANCE OF DENITRIFICATION

A key process in the N cycle is denitrification, which is a heterotrophic process performed by facultative anaerobic organisms that utilize various C substrates as electron donors (Beauchamp *et al.* 1989; Davidson & Seitzinger 2006). Therefore a better understanding of this process and the dynamics of associated N transformations is pivotal. Nitrate (NO_3^-) is the key N species for N losses and is affected by numerous N flows that produce (e.g. nitrification) and consume (e.g. leaching) NO_3^- (Fig. 3). To be rendered environmentally benign, NO_3^- must be reduced to a non-reactive form (e.g. dinitrogen, N_2). Three major biological pathways of NO_3^- reduction are known (Fig. 3): (i) assimilatory NO_3^- reduction into biomass, (ii) dissimilatory NO_3^- reduction to NH_4^+ (DNRA) and (iii) dissimilatory NO_3^- reduction to N_2 (denitrification)

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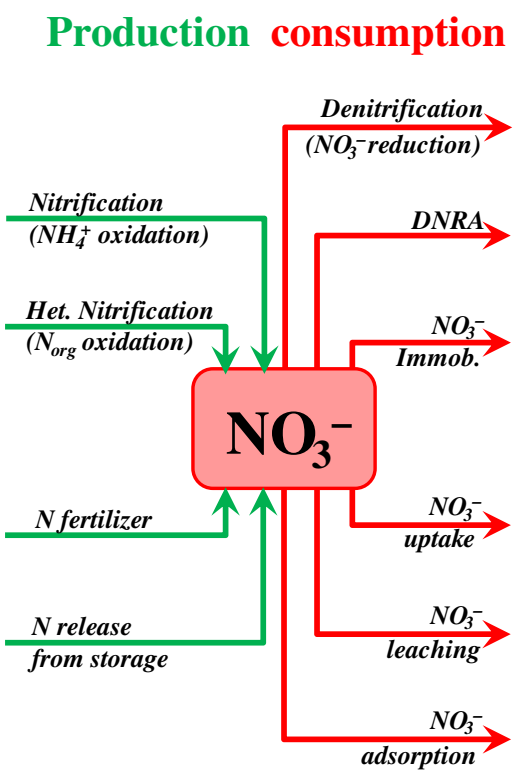


Fig. 3. Major production and consumption processes affecting the size of the soil NO_3^- pool size (note the interactions of NO_3^- with a hypothetical storage component follows the ideas of Müller *et al.* (2002) (colour version available online).

(Robertson & Kuenen 1990; Burger & Jackson 2005). Dissimilatory reduction may outcompete denitrification under conditions where electron acceptors are limited and it provides an energetically favourable alternative to denitrification (Tiedje 1988; Rütting *et al.* 2011). However, further research is still required to understand the importance of DNRA in terrestrial systems and to perfect methods for studying the process (Rütting *et al.* 2011).

Denitrification is a key transformation process in soils with both adverse and beneficial roles, since it is both a source and sink for N_2O, impairs the N use efficiency of agricultural crops and lowers the potential for NO_3^- leaching to aquatic systems (Davidson & Seitzinger 2006). In aquatic systems, autotrophic denitrification may also occur in the presence of inorganic electron donors such as sulphides or ferrous iron (Knowles 1982; Clément *et al.* 2005).

The reductases involved in denitrification are well recognized. The membrane-bound nitrate reductase (*Nar*) is the first in the denitrification sequence and occurs in very diverse microbial communities including α, β, γ and ε proteobacteria, Gram-positive bacteria and archaea, while the periplasmic NO_3^- reductase (*Nap*) occurs only in Gram-negative bacteria (Philippot 2005). The key enzyme and key precursor for gaseous N emissions in the denitrification sequence is nitrite reductase that is encoded by either *nirS*, a cytochrome cd1-containing gene, or *nirK*, a copper-containing gene. The two nitrite reductases provide a functional marker for the diversity of denitrifying bacteria (Braker *et al.* 2000; Braker & Conrad 2011). The genes *norB* and *nosZ* encode NO and N_2O reductase, respectively (Groffman *et al.* 2006) and it is the dynamics of these two reductases in combination with substrate and environmental regulators and in particular the balance between *nar/nir* and *nos* that controls the net production and release of N_2O. Philippot *et al.* (2011) showed that denitrifiers with and without *nosZ* encoding can co-exist and that higher N_2O/N_2 ratios can be related to the proportions of bacteria with and without *nosZ* encoding. Differential encoding may also be related to soil pH, which may differ in distinct niches (Philippot *et al.* 2009). This highlights the fact that the observed overall denitrification dynamic is governed by the sum of the individual dynamics of co-existing microbes, possibly living in different niches (Philippot *et al.* 2011) and/or soil aggregates (Miller *et al.* 2009). Moreover, aggregation affects diffusive exchange of mineral (e.g. NO_3^-) and gaseous N species (e.g. N_2O) and O_2 between denitrifying microsite and inter-aggregate pores, which affects both total denitrification (Sexstone *et al.* 1985) and the product ratio of denitrification (Arah & Smith 1989).

While available organic C and O_2 have a major impact on total denitrification, soil pH mainly influences the ratio of N_2O/N_2 (Šimek & Cooper 2002). Maximum denitrification occurs usually at relatively high pH value and a linear relationship is often observed between denitrification and pH (Focht 1974). There is a tendency for the N_2O/N_2 ratio to increase with decreasing pH (Burford & Bremner 1975; Šimek & Cooper 2002; Liu *et al.* 2010) and a more complete reduction towards N_2 is found at higher pH values (Nõmmik 1956). The effect of pH may be due to an impact on substrate availability that influences membrane permeability or speciation of chemical species (Dassonville & Renault 2002). Nitrous oxide reductase is more sensitive to pH than other reductases in the denitrification sequence and therefore low pH may increase the lag phase of *de novo* synthesis of this enzyme (Šimek & Cooper 2002; Liu *et al.* 2010). Apparently the detrimental effect of

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low pH on N_2O reductase occurs at the post-transcriptional level by interfering with translation, protein assembly or an effect on the activity of functional enzymes (Liu *et al*. 2010). Microsite variations in soil pH may support a diverse microbial community that exhibits a different sensitivity to pH (Cavigelli & Robertson 2000; Šimek & Cooper 2002). Also, chemodenitrification may play a role for N_2O and N_2 production in acidic microsites (Chalk & Smith 1983; Stevens *et al*. 1998). At pH < 3, chemical decomposition of NO_2^- is most likely to be the dominant process for N_2 production and may account for up 0·5 of N losses in tropical soils (Laudelout *et al*. 1977). Denitrifiers adapt to low pH over long periods of time; thus experimental setups including *ad hoc* pH manipulations are not conducive to evaluating natural soil conditions (Šimek & Cooper 2002). However, one of the key unknowns is the lack of suitable field methods to quantify the N_2O/N_2 ratio *in situ*. Techniques based on the inhibition of acetylene are no longer appropriate (Bollmann & Conrad 1997) and easy-to-use ^{15}N labelling techniques are still lacking and can be prohibitively expensive.

In general, it is the relationship between microbial communities in diverse ecosystems, the numerous proximal and distal factors and ecosystem functioning, which are not yet completely understood (Braker & Conrad 2011). In particular, the activities of microbial communities under various conditions and how these contribute to soil N dynamics is a very important area of research. Studies such as Jørgensen *et al*. (2012), which examined gaseous N losses at spatial and temporal scales and the factors affecting these, make important contributions to elucidating the detail of the processes and pathways surrounding ecosystem N dynamics *in situ*.

UNDERSTANDING N DYNAMICS AND THE ASSOCIATED MICROBIAL COMMUNITIES

Microbial community dynamics are influenced by the interacting effects of environmental conditions together with the substrate relationships (carbon substrates) and the availability of electron acceptors (N-oxides). For instance, the most important environmental regulators for denitrification are: oxygen > pH > temperature (Focht 1974). Mineralization activity provides substrates and is closely related to moisture/oxygen–temperature conditions (Focht & Verstraete 1977; Myrold & Tiedje 1985*b*; Morley & Baggs 2010). A high rate of C mineralization favours oxygen (O) consumption. However, in fertilized soils, C rather than NO_3^- availability limits total denitrification, but NO_3^- has a pronounced effect on the ratio of N_2O/N_2 (Nõmmik 1956; Myrold & Tiedje 1985*a*, *b*; Beauchamp *et al*. 1989; McCarty & Bremner 1993; Dendooven & Anderson 1994). Many studies have focused on either proximal or distal factors, as described by Groffman *et al*. (1988), but only a few studies so far have tried to link these factors. The C substrate determines the efficiency with which N oxides (NO_3^-, NO_2^-) are reduced (Beauchamp *et al*. 1989). Denitrifiers are able to compete successfully for C with other heterotrophs (Myrold & Tiedje 1985*b*). Thus, specific microbe–substrate relationships exist that may explain the link between decomposition products and microbial populations (Beauchamp *et al*. 1989). However, these relationships are still not well understood. The availability and identity of the C substrates and in particular the ratio between C substrate to e^--acceptor also governs whether denitrification or DNRA occurs in soils. Although the need for C is universally acknowledged, there is still a need to identify assays that can predict forms of C substrate utilized by various microbial groups (Rütting *et al*. 2011) in various soil niches. Thus, to advance the mechanistic understanding of microbial N cycling in terrestrial ecosystems there is also a need to link microsite and not only bulk soil conditions to microbial activities.

In natural environments diverse microbial populations are observed (Tiedje 1988). The greatest spatial aggregation is generally observed in the topsoil (Nunan *et al*. 2002) and is most probably a response to the patchy distribution of organic material (Parkin 1987) and environmental conditions (Tiedje 1988) such as aerobic–anaerobic microsites. The need to better understand the microenvironment where N transformations occur was emphasized more than 40 years ago (McLaren 1970) but the progress in this area has been slow. Microbial processes are affected by substrate availability, thermodynamic regulation and inhibition, pH and redox potential (Brock 1967; Firestone & Davidson 1989; Dassonville & Renault 2002). Interactions between these factors are not fully understood. Cavigelli & Robertson (2000) investigated for instance the effect of pH and oxygen on the N_2O/N_2 ratio in both an arable and non-disturbed successional field, and observed that differences were most probably governed by the prevailing microbial community.

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NEW CONCEPTUAL FRAMEWORKS AND MODELLING CONCEPTS FOR SOIL N DYNAMICS

There is a need for new theoretical and conceptual frameworks to understand how microorganisms influence ecosystem processes (Hall *et al*. 2011). Two aspects need to be taken into account to understand the relationships between environmental regulators and microbial activity. First, there needs to be an acknowledgement that different *microsites* exist in the soil, which to date have been considered as homogenous spaces with respect to environmental regulators. Secondly, there is a requirement to identify the *units* that represent physiologically similar microbial entities that live in mutualistic or antagonistic relationships within these *microsites*. For instance, while anaerobic conditions are required for denitrification to proceed, aerobic denitrification (NO_3^- reduction under microaerophyillic conditions) has been observed (Bréal 1892). This latter process seems to be related to heterotrophic nitrification and most probably presents a detoxifying mechanism to reduce high NO_2^- concentrations (Robertson & Kuenen 1990).

Thus, to understand gaseous N dynamics from terrestrial ecosystems, it is essential to understand the individual potential production processes. For instance, N_2O is produced by nitrifiers and denitrifiers and also via groups that combine the two processes via nitrifier–denitrification (Wrage *et al*. 2001). Kool *et al*. (2011) showed that the nitrifier–denitrification pathway can be a major source for N_2O if conditions for denitrification are suboptimal and therefore it should not be ignored as a source of N_2O from soil (Wrage *et al*. 2001).

Furthermore, chemical transformations may also contribute to N dynamics. Chemodenitrification may occur in low pH soils, including the van Slyke reaction where organic N together with NO_2^- reacts to form N_2 (Tiedje 1988). However, understanding of the potential contribution of chemodenitrification to gaseous N losses as NO and N_2O is far from complete, as demonstrated by recent studies. Further studies on chemodenitrification are warranted, especially under high N input agricultural systems (Spott *et al*. 2011). Besides bacteria, fungi and archaea are capable of N transformations such as NO_3^- assimilation and denitrification when NH_4^+ is depleted (Zumft 1997; Laughlin & Stevens 2002). However, quantitative understanding of both the fungal denitrification (Morozkina & Kurakov 2007) and archaeal N transformation processes are still in their infancy.

Modelling concepts

An important tool to simultaneously consider all N cycling processes is mathematical modelling. Betlach & Tiedje (1981) concluded that kinetic models can provide a useful guide to evaluate the physiology of microbial N transformations. Various kinetic expressions such as zero-order, first-order or Michaelis–Menten kinetics can be tested easily as well as dual-substrate relationships (Bowman & Focht 1974; Focht 1974; Betlach & Tiedje 1981). In kinetic chain reactions such as denitrification the N dynamics can be governed by the dynamics of electron donors and N oxides for each reduction step and competition between organisms for NO_3^-, which is governed by C availability (Dendooven *et al*. 1994).

Diffusion and conditions in aggregated soil

Kinetic parameters in N cycling models that are applied to field situations are probably not representative of the true kinetic reaction at the reaction sites because heterogenic distribution and diffusion of N oxides and electron donors has to be taken into account (Focht 1974; Betlach & Tiedje 1981). In aggregated soils it is important to consider diffusion to the centre of aggregates where denitrification predominantly occurs (a kind of 'hot spot' for denitrification) (Arah 1990*b*). Therefore microsite-specific conditions and microbial interactions need to be taken into account (Myrold & Tiedje 1985*a*; Arah 1990*b*). In particular, the distribution of microbes and their activity should be included across the range of soil aggregates (Miller *et al*. 2009). In diffusion–reaction models that take into account the aeration status, the diffusion of oxygen (Greenwood & Goodman 1964; McElwain 1978) and of the various N species are required to predict N cycling in structured soils (Burford & Stefanson 1973; Arah 1990*a*).

General model development

Models for microbial N dynamics should be embedded in larger models that predict microbial processes and their link to nutrient cycling and take into account the high spatial and temporal nature of these processes (Groffman 1991). Besides simple relationships, the complexity of the microbial

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reactions that reflect the high spatial and temporal variability need to be considered to adequately connect ecological theory with molecular–microbial composition and function (Groffman 1991). Thus spatial and temporal explicit relationships between, for example, the denitrifier community and environmental regulators should be taken into account. Furthermore, a bottom-up approach should be considered where spatial and temporal variability of microbial dynamics at the microsite level will be used to predict N flows and transformations at the field and regional scale. Furthermore, modelling concepts should be embedded in models that simulate the interactions among the various elemental cycles such as C–N–P (e.g. NCSOIL, Molina *et al.* 1983; CENTURY, Parton *et al.* 1987; DNDC, Li *et al.* 1992). Currently no model exists that takes into account all these aspects, in particular the high spatial and temporal nature of microbial processes.

ADVANCES IN ANALYTICAL APPROACHES – RESOLUTION OF SMALL SCALES

Mathematical models are only as good as the dataset addressing the various model components for model development and validation. While mathematical concepts regarding small-scale diffusion processes were already well advanced more than 20 years ago (Bouldin 1961; Misra *et al.* 1974; McConnaughey & Bouldin 1985; Arah 1990*a*), it is only now possible to validate them with appropriate techniques that are able to resolve the small physical scales (Herrmann *et al.* 2007; Vogel *et al.* 2010).

Stable isotope techniques (^{13}C, ^{15}N) in combination with suitable mathematical analysis tools are available to quantify simultaneously occurring N transformations dynamics in soil. With the latest ^{15}N tracing models it is possible to identify more than 14 simultaneously occurring N transformations including nitrite dynamics (Rütting & Müller 2008) and plant uptake dynamics (Inselsbacher *et al.* 2013). Together with the latest analytical techniques using nanoSIMS, it should be possible to resolve processes at µm or nm scales and the gross N dynamics at these fine spatial scales (Herrmann *et al.* 2007; Clode *et al.* 2009). This will most probably alter the current view of soil N dynamics because of the non-linearity among microsites (i.e. a linear relationship between microsites is inherently assumed with bulk soil determinations).

At the µm and mm scales, advanced microsensor techniques are available for a range of gaseous and mineral N species (Andersen *et al.* 2001; Meyer *et al.* 2002; Neumann *et al.* 2009), which were recently applied in field studies (Jørgensen *et al.* 2012). Non-invasive techniques, such as X-ray computed tomography (CT), allow three-dimensional visualization of soil structures and pore geometry at µm scales (Wildenschild *et al.* 2002; Vogel *et al.* 2010). Together with techniques such as microdialysis (Inselsbacher *et al.* 2011), it will be possible to resolve N transformations at small scales in the field, which will without doubt generate data that reflect much more realistically the N transformation processes in structured soils.

CONCLUSIONS

The advancement of knowledge regarding N flows and transformations hinges on the identification of N dynamics at small scales. A combination of suitable analytical techniques with appropriate modelling will help future understanding of spatial heterogeneity in soil-plant and aggregated systems.

We thank the organisers of the 17th N workshop who invited us to present this keynote presentation. We acknowledge financial support from the OECD.

REFERENCES

Ackert, L. T. Jr. (2006). The role of microbes in agriculture: Sergei Vinogradskii's discovery and investigation of chemosynthesis, 1880–1910. *Journal of the History of Biology* **39**, 373–406.

Andersen, K., Kjær, T. & Revsbech, N. P. (2001). An oxygen insensitive microsensor for nitrous oxide. *Sensors and Actuators B: Chemical* **81**, 42–48.

Arah, J. & Smith, K. A. (1989). Steady-state denitrification in aggregated soils: a mathematical model. *Journal of Soil Science* **40**, 139–149.

Arah, J. R. M. (1990*a*). Diffusion–reaction models of denitrification in soil microsites. In *Denitrification in Soil and Sediment* (Eds N. P. Revsbech & J. Sørensen), pp. 245–258. New York: Plenum Press.

Arah, J. R. M. (1990*b*). Modelling spatial and temporal variability of dentrification. *Biology and Fertility of Soils* **9**, 71–77.

Aulie, R. P. (1970). Boussingault and the nitrogen cycle. *Proceedings of the American Philosophical Society* **114**, 435–479.

Barnard, R., Leadley, P. W., Lensi, R. & Barthes, L. (2005). Plant, soil microbial and soil inorganic nitrogen responses to elevated CO_2: a study in microcosms of *Holcus lanatus*. *Acta Oecologica* **27**, 171–178.

https://doi.org/10.1017/S0021859613000610 Published online by Cambridge University Press

Barnosky, A. D., Hadly, E. A., Bascompte, J., Berlow, E. L., Brown, J. H., Fortelius, M., Getz, W. M., Harte, J., Hastings, A., Marquet, P. A., Martinez, N. D., Mooers, A., Roopnarine, P., Vermeij, G., Williams, J. W., Gillespie, R., Kitzes, J., Marshall, C., Matzke, N., Mindell, D. P., Revilla, E. & Smith, A. B. (2012). Approaching a state shift in Earth's biosphere. *Nature* **486**, 52–58.

Beauchamp, E. G., Trevors, J. T. & Paul, J. W. (1989). Carbon sources for bacterial denitrification. *Advances in Soil Science* **10**, 113–142.

Betlach, M. R. & Tiedje, J. M. (1981). Kinetic explanation for accumulation of nitrite, nitric oxide, and nitrous oxide during bacterial denitrification. *Applied and Environmental Microbiology* **42**, 1074–1084.

Bollmann, A. & Conrad, R. (1997). Acetylene blockage technique leads to underestimation of denitrification rates in oxic soils due to scavenging of intermediate nitric oxide. *Soil Biology and Biochemistry* **29**, 1067–1077.

Bouldin, D. R. (1961). Mathematical description of diffusion processes in the soil–plant system. *Soil Science Society of America Journal* **25**, 476–480.

Bowman, R. A. & Focht, D. D. (1974). The influence of glucose and nitrate concentrations upon denitrification rates in sandy soils. *Soil Biology and Biochemistry* **6**, 297–301.

Braker, G. & Conrad, R. (2011). Diversity, structure, and size of N_2O-producing microbial communities in soils – What matters for their functioning? *Advances in Applied Microbiology* **75**, 33–70.

Braker, G., Zhou, J., Wu, L., Devol, A. H. & Tiedje, J. M. (2000). Nitrite reductase genes (*nirK* and *nirS*) as functional markers to investigate diversity of denitrifying bacteria in Pacific Northwest Marine Sediment Communities. *Applied and Environmental Microbiology* **66**, 2096–2104.

Bréal, E. (1892). De la prèsence, dans la paille, d'un ferment aérobie, réducteur des nitrates. *Comptes Rendus hebdomadaires des Séance de l'Academie des Sciences (Paris)* **114**, 681–684.

Brock, T. D. (1967). The ecosystem and the steady state. *BioScience* **17**, 166–169.

Burford, J. R. & Bremner, J. M. (1975). Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Biology and Biochemistry* **7**, 389–394.

Burford, J. R. & Stefanson, R. C. (1973). Measurements of gaseous losses of nitrogen from soils. *Soil Biology and Biochemistry* **5**, 133–141.

Burger, M. & Jackson, L. E. (2005). Plant and microbial use and turnover: rapid conversion of nitrate to ammonium in soil with roots. *Plant and Soil* **266**, 289–301.

Cabrera, M. L., Kissel, D. E. & Vigil, M. F. (2005). Nitrogen mineralization from organic residues: research opportunities. *Journal of Environmental Quality* **34**, 75–79.

Cavigelli, M. A. & Robertson, G. P. (2000). The functional significance of denitrifier community composition in a terrestrial ecosystem. *Ecology* **81**, 1402–1414.

Chalk, P. M. & Smith, C. J. (1983). Chemodenitrification. In *Gaseous Loss of Nitrogen from Plant Soil Systems* (Eds J. R. Freney & J. R. Simpson), pp. 65–89. Developments in Plant and Soil Sciences 9. Dordrecht: Martinus Nijhoff and Dr W Junk.

Clément, J.-C., Shrestha, J., Ehrenfeld, J. G. & Jaffé, P. R. (2005). Ammonium oxidation coupled to dissimilatory reduction of iron under anaerobic conditions in wetland soils. *Soil Biology and Biochemistry* **37**, 2323–2328.

Clode, P. L., Kilburn, M. R., Jones, D. L., Stockdale, E. A., Cliff, J. B. III, Herrmann, A. M. & Murphy, D. V. (2009). *In situ* mapping of nutrient uptake in the rhizosphere using nanoscale secondary ion mass spectrometry. *Plant Physiology* **151**, 1751–1757.

Dassonville, F. & Renault, P. (2002). Interactions between microbial processes and geochemical transformations under anaerobic conditions: a review. *Agronomie* **22**, 51–68.

Davidson, E. A. & Seitzinger, S. (2006). The enigma of progress in dentrification research. *Ecological Applications* **16**, 2057–2063.

Deangelis, K. M., Lindow, S. E. & Firestone, M. K. (2008). Bacterial quorum sensing and nitrogen cycling in rhizosphere soil. *FEMS Microbiology Ecology* **66**, 197–207.

Dendooven, L. & Anderson, J. M. (1994). Dynamics of reduction enzymes involved in the denitrification process in pasture soil. *Soil Biology and Biochemistry* **26**, 1501–1506.

Dendooven, L., Splatt, P., Anderson, J. M. & Scholefield, D. (1994). Kinetics of the denitrification process in a soil under permanent pasture. *Soil Biology and Biochemistry* **26**, 361–370.

Firestone, M. K. & Davidson, E. A. (1989). Microbiological basis of NO and N_2O production and consumption in soil. In *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere* (Eds M. O. Andreae & D. S. Schimel), pp. 7–21. New York: John Wiley & Sons.

Focht, D. D. (1974). The effect of temperature, pH, and aeration on the production of nitrous oxide and gaseous nitrogen – a zero order kinetic model. *Soil Science* **118**, 173–179.

Focht, D. D. & Verstraete, E. (1977). Biochemical ecology of nitrification and denitrification. In *Advances in Microbial Ecology* (Ed. M. Alexander), pp. 135–214. New York: Plenum Press.

Galloway, J. N. & Cowling, E. B. (2002). Reactive nitrogen and the world: 200 years of change. *Ambio* **31**, 64–71.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R. & Vörösmarty, C. J. (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry* **70**, 153–226.

Galloway, J. N., Townsend, A. R., Erisman, J. W., Bekunda, M., Cai, Z., Freney, J. R., Martinelli, L. A., Seitzinger, S. P. & Sutton, M. A. (2008). Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* **320**, 889–893.

https://doi.org/10.1017/S0021859613000610 Published online by Cambridge University Press

Greenwood, D. J. & Goodman, D. (1964). Oxygen diffusion and aerobic respiration in soil spheres. *Journal of the Science of Food and Agriculture* **15**, 579–588.

Groffman, P. M. (1991). Ecology of nitrification and denitrification in soil evaluated at scales relevant to atmospheric chemistry. In *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes* (Eds J. E. Rogers & W. B. Whitman), pp. 201–217. Washington, DC: American Society for Microbiology.

Groffman, P. M., Tiedje, J. M., Robertson, G. P. & Christensen, S. (1988). Denitrification at different temporal and geographical scales: proximal and distal controls. In *Advances in Nitrogen Cycling in Agricultural Ecosystems* (Ed. J. R. Wilson), pp. 174–192. Wallingford: CAB International.

Groffman, P. M., Altabet, M. A., Böhlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., Kana, T. M., Nielsen, L. P. & Voytek, M. A. (2006). Methods for measuring denitrification diverse approaches to a difficult problem. *Ecological Applications* **16**, 2091–2122.

Hall, E. K., Maixner, F., Franklin, O., Daims, H., Richter, A. & Battin, T. (2011). Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. *Ecosystems* **14**, 261–273.

Herrmann, A. M., Ritz, K., Nunan, N., Clode, P. L., Pett-Ridge, J., Kilburn, M. R., Murphy, D. V., O'donnell, A. G. & Stockdale, E. A. (2007). Nano-scale secondary ion mass spectrometry – a new analytical tool in biogeochemistry and soil ecology: a review article. *Soil Biology and Biochemistry* **39**, 1835–1850.

Inselsbacher, E., Öhlund, J., Jämtgard, S., Huss-Danell, K. & Näsholm, T. (2011). The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. *Soil Biology and Biochemistry* **43**, 1321–1332.

Inselsbacher, E., Wanek, W., Strauss, J., Zechmeister-Boltenstern, S. & Müller, C. (2013). A novel ^{15}N tracer model reveals: plant nitrate uptake governs nitrogen transformation rates in agricultural soils. *Soil Biology and Biochemistry* **57**, 301–310.

Jones, D. L., Shannon, D., Murphy, D. V. & Farrar, J. (2004). Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. *Soil Biology and Biochemistry* **36**, 749–756.

Jørgensen, C. J., Struwe, S. & Elberling, B. (2012). Temporal trends in N_2O flux dynamics in a Danish wetland – effects of plant-mediated gas transport of N_2O and O_2 following changes in water level and soil mineral-N availability. *Global Change Biology* **18**, 210–222.

Knops, J. M. H., Bradley, K. L. & Wedin, D. A. (2002). Mechanisms of plant-species impacts on ecosystem nitrogen cycling. *Ecology Letters* **5**, 454–466.

Knowles, R. (1982). Denitrification. *Microbiological Reviews* **46**, 43–70.

Kool, D. M., Dolfing, J., Wrage, N. & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry* **43**, 174–178.

Laudelout, H., Germain, L., Chabalier, P. F. & Chiang, C. N. (1977). Computer simulation of loss of fertilizer nitrogen through chemical decomposition of nitrite. *Journal of Soil Science* **28**, 329–339.

Laughlin, R. J. & Stevens, R. J. (2002). Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. *Soil Science Society of America Journal* **66**, 1540–1548.

Li, C., Frolking, S. & Frolking, T. A. (1992). A model of nitrous oxide evolution from soil driven by rainfall events: 1. model structure and sensitivity. *Journal of Geophysical Research: Atmospheres* **97**, 9759–9776.

Liu, B., Mørkved, P. T., Frostegård, Å. & Bakken, L. R. (2010). Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 productions affected by soil pH. *FEMS Microbiology Ecology* **72**, 407–417.

Manzoni, S., Jackson, R. B., Trofymow, J. A. & Porporato, A. (2008). The global stoichiometry of litter nitrogen mineralization. *Science* **321**, 684–686.

Mccarty, G. W. & Bremner, J. M. (1993). Factors affecting the availabiltiy of organic carbon for denitrification of nitrate in subsoils. *Biology and Fertility of Soils* **15**, 132–136.

Mcconnaughey, P. K. & Bouldin, D. R. (1985). Transient microsite models of denitrification. I. Model development. *Soil Science Society of America Journal* **49**, 886–891.

Mcelwain, D. L. S. (1978). A re-examination of oxygen diffusion in a spherical cell with Michaelis–Menten oxygen uptake kinetics. *Journal of Theoretical Biology* **71**, 255–263.

Mclaren, A. D. (1970). Temporal and vectorial reactions of nitrogen in soil: a review. *Canadian Journal of Soil Science* **50**, 97–109.

Meyer, R. L., Kjær, T. & Revsbech, N. P. (2002). Nitrification and denitrification near a soil–manure interface studied with a nitrate–nitrite biosensor. *Soil Science Society of America Journal* **66**, 498–506.

Miller, M. N., Zebarth, B. J., Dandie, C. E., Burton, D. L., Goyer, C. & Trevors, J. T. (2009). Denitrifier community dynamics in soil aggregates under permanent grassland and arable cropping systems. *Soil Science Society of America Journal* **73**, 1843–1851.

Misra, C., Nielsen, D. R. & Biggar, J. W. (1974). Nitrogen transformations in soil during leaching. I. Theoretical considerations. *Soil Science Society of America Proceedings* **38**, 289–293.

Molina, J. A. E., Clapp, C. E., Shaffer, M. J., Chichester, F. W. & Larson, W. E. (1983). NCSOIL, A model of nitrogen and carbon transformations in soil: description, calibration, and behavior. *Soil Science Society of America Journal* **47**, 85–91.

Morley, N. & Baggs, E. M. (2010). Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction. *Soil Biology and Biochemistry* **42**, 1864–1871.

Morozkina, E. V. & Kurakov, A. V. (2007). Dissimilatory nitrate reduction in fungi under conditions of hypoxia and

https://doi.org/10.1017/S0021859613000610 Published online by Cambridge University Press

anoxia: a review. *Applied Biochemistry and Microbiology* **43**, 544–549.

Müller, C., Martin, M., Stevens, R. J., Laughlin, R. J., Kammann, C., Ottow, J. C. G. & Jäger, H.-J. (2002). Processes leading to N_2O emissions in grassland soil during freezing and thawing. *Soil Biology and Biochemistry* **34**, 1325–1331.

Myrold, D. D. & Tiedje, J. M. (1985*a*). Diffusional constraints on denitrification in soil. *Soil Science Society of America Journal* **49**, 651–657.

Myrold, D. D. & Tiedje, J. M. (1985*b*). Establishment of denitrification capacity in soil: effects of carbon, nitrate and moisture. *Soil Biology and Biochemistry* **17**, 819–822.

Neumann, G., George, T. S. & Plassard, C. (2009). Strategies and methods for studying the rhizosphere – the plant science toolbox. *Plant and Soil* **321**, 431–456.

Nõmmik, H. (1956). Investigations on denitrification in soil. *Acta Agriculturae Scandinavica* **6**, 195–228.

Nunan, N., Wu, K., Young, I. M., Crawford, J. W. & Ritz, K. (2002). *In situ* spatial patterns of soil bacterial populations, mapped at multiple scales, in an arable soil. *Microbial Ecology* **44**, 296–305.

Osler, G. H. R. & Sommerkorn, M. (2007). Toward a complete soil C and N cycle: incorporating the soil fauna. *Ecology* **88**, 1611–1621.

Parkin, T. B. (1987). Soil microsites as a source of denitrification variability. *Soil Science Society of America Journal* **51**, 1194–1199.

Parton, W., Silver, W. L., Burke, I. C., Grassens, L., Harmon, M. E., Currie, W. S., King, J. Y., Adair, E. C., Brandt, L. A., Hart, S. C. & Fasth, B. (2007). Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* **315**, 361–364.

Parton, W. J., Schimel, D. S., Cole, C. V. & Ojima, D. S. (1987). Analysis of factors controlling soil organic matter levels in great plains grasslands. *Soil Science Society of America Journal* **51**, 1173–1179.

Philippot, L. (2005). Tracking nitrate reducers and denitrifiers in the environment. *Biochemical Society Transactions* **33**, 200–204.

Philippot, L., Čuhel, J., Saby, N. P. A., Chèneby, D., Chroňáková, A., Bru, D., Arrouays, D., Martin-Laurent, F. & Šimek, M. (2009). Mapping field-scale spatial patterns of size and activity of the denitrifier community. *Environmental Microbiology* **11**, 1518–1526.

Philippot, L., Andert, J., Jones, C. M., Bru, D. & Hallin, S. (2011). Importance of denitrifiers lacking the genes encoding the nitrous oxide reductase for N_2O emissions from soil. *Global Change Biology* **17**, 1497–1504.

Rastetter, E. B., Ågren, G. I. & Shaver, G. R. (1997). Responses of N-limited ecosystems to increased CO_2: a balanced-nutrition, coupled-element-cycles model. *Ecological Applications* **7**, 444–460.

Robertson, L. A. & Kuenen, J. G. (1990). Physiological and ecological aspects of aerobic denitrification. In *Denitrification in Soil and Sediment* (Eds N. P. Revsbech & J. Sørensen), pp. 91–104. New York: Plenum Press.

Rütting, T. & Müller, C. (2008). Process-specific analysis of nitrite dynamics in a permanent grassland soil by using a Monte Carlo sampling technique. *European Journal of Soil Science* **59**, 208–215.

Rütting, T., Boeckx, P., Müller, C. & Klemedtsson, L. (2011). Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle. *Biogeosciences* **8**, 1779–1791.

Sanderman, J. & Amundson, R. (2003). Biogeochemistry of decomposition and detrital processing. In *Biogeochemistry* (Ed. W. H. Schlesinger), pp. 249–316. Treatise on Geochemistry vol. 8. Amsterdam: Elsevier.

Schimel, J. P. & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology* **85**, 591–602.

Sexstone, A. J., Revsbech, N. P., Parkin, T. B. & Tiedje, J. M. (1985). Direct measurement of oxygen profiles and denitrification rates in soil aggregates. *Soil Science Society of America Journal* **49**, 645–651.

Šimek, M. & Cooper, J. E. (2002). The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science* **53**, 345–354.

Spott, O., Russow, R. & Stange, C. F. (2011). Formation of hybrid N_2O and hybrid N_2 due to codenitrification: first review of a barely considered process of microbially mediated N-nitrosation. *Soil Biology and Biochemistry* **43**, 1995–2011.

Stevens, R. J., Laughlin, R. J. & Malone, J. P. (1998). Soil pH affects the processes reducing nitrate to nitrous oxide and di-nitrogen. *Soil Biology and Biochemistry* **30**, 1119–1126.

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchia, N., Jenkins, M., Minasny, B., Mcbratney, A. B., De Remy De Courcelles, V., Singh, K., Wheeler, I., Abbott, L., Angers, D. A., Baldock, J., Bird, M., Brookes, B. C., Chenu, C., Jastrow, J. D., Lal, R., Lehmann, J., O'donnell, A. G., Parton, W. J., Whitehead, D. & Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture, Ecosystems and Environment* **164**, 80–99.

Tiedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In *Biology of Anaerobic Microorganisms* (Ed. A. J. B. Zehnder), pp. 179–244. New York: John Wiley & Sons.

Uchida, Y., Clough, T. J., Kelliher, F. M., Hunt, J. E. & Sherlock, R. R. (2011). Effects of bovine urine, plants and temperature on N_2O and CO_2 emissions from a sub-tropical soil. *Plant and Soil* **345**, 171–186.

Van Der Ploeg, R. R., Böhm, W. & Kirkham, M. B. (1999). On the origin of the theory of mineral nutrition of plants and the law of the minimum. *Soil Science Society of America Journal* **63**, 1055–1062.

Van Kessel, C., Clough, T. J. & Van Groenigen, J. W. (2009). Dissolved organic nitrogen: an overlooked pathway of nitrogen loss from agricultural systems? *Journal of Environmental Quality* **38**, 393–401.

Vogel, H.-J., Weller, U. & Schlüter, S. (2010). Quantification of soil structure based on Minkowski functions. *Computers and Geosciences* **36**, 1236–1245.

Wildenschild, D., Hopmans, J. W., Vaz, C. M. P., Rivers, M. L., Rikard, D. & Christensen, B. S. B. (2002). Using X-ray

https://doi.org/10.1017/S0021859613000610 Published online by Cambridge University Press

computed tomography in hydrology: systems, resolutions, and limitations. *Journal of Hydrology* **267**, 285–297.

Wrage, N., Velthof, G. L., Van Beusichem, M. L. & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry* **33**, 1723–1732.

Zak, D. R., Holmes, W. E., White, D. C., Peacock, A. D. & Tilman, D. (2003). Plant diversity, soil microbial communities, and ecosystem function: are there any links? *Ecology* **84**, 2042–2050.

Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews* **61**, 533–616.

https://doi.org/10.1017/S0021859613000610 Published online by Cambridge University Press