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Interactive comment on "The soil N cycle: new insights and key challenges" *by* J. W. van Groenigen et al.

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J. W. van Groenigen et al.: The soil N cycle. New insights and key challenges

This review summarizes "insights made over the last decade" and gives a "personal view on key challenges" of the soil N cycle. Four challenges are presented upfront in the abstract, each of which is linked to a specific N cycle process (none-symbiotic N fixation, nitrifier denitrification, microbial N2O consumption, and denitrification). This is followed by three groups of organisms (soil fauna, roots and mycorrhyzal symbionts) exerting proximal control on soil N cycling. The abstract is wrapped up by saying that better 15N and 18O tracing models are essential for further advancing our knowledge on the N cycle by disentangling gross transformation rates.

The manuscript gives some exiting insights into the multiple research fronts of soil N

cycling. The trade-off is its lack in conceptual coherence. The choice of key issues represents the "personal views" of the authors (627, L. 4) and there is little attempt to place these key-challenges into a heuristic context. For instance, the introduction gives the impression that watershed biogeochemistry and N budgeting are the guiding principles for this review (626, L. 11 ff), which is not the case, given the nature of the various identified key-issues: key-question 1 comes along primarily as a biogeochemical one (although it contains many microbial ecology questions), key-question 2 relates to biochemistry and physiology (even though it is framed mainly as a methodological problem), key-question 3 relates both to biochemistry and ecolology, whereas key-question 4 is primarily a methodological one. Figure 1 places these challenges correctly on the N cycle map, but it does not tell why, how and to what end these issues have been selected. Probably a more functional approach like that given in figure 3 of Osobe and Ohte (2014) would help. In any case, more precision in argument is needed in the introduction to justify the selection. For instance, if a pathway is illusive (627, L. 12), how can we know whether it is relevant? There might be good reasons, but then give reason here. Or, why exactly is it important to capture hot-spots and hot-moments in denitrification? Spell it out! Thus, the introduction has potential for improvement.

Since it is the authors' intention is to stimulate an educated debate on an "N research agenda" to come for the next decade (627, L. 24), I will organize my evaluation along the following two questions:

1. Do the chapters elaborate sufficiently on why the chosen processes hold key-challenges to our (ecological) understanding of the soil N-cycle?

2. Are the reasons/insights given sufficient to justify the choice of a specific "key-challenge" within each process/controll?

Emerging insights 1 – N2 fixation

The text is well written, and it becomes immediately clear that better knowledge on N fixating organisms and processes in natural ecosystems is needed to predict ecosystem responses to global change. This topic is well justified. It remains somewhat unclear which methodological approaches the authors recommend to achieve this goal. Direct 15N2 labelling seems to be preferable over acetylene reduction, and more spatially explicit data are needed (629, L. 9-11). Above this, the diversity, niches and nutrient controls of free-living diazotrophs seem to be unclear. Smart manipulation experiments will be needed to fully elucidate that. Some more methodological outline could improve this chapter.

Emerging insights 2 – nitrifier denitrification

I agree that there has been a problem with terminology. I never understood why N2O production during nitrification is not simply distinguished on the basis of the oxidative or reductive nature of its biochemical formation. Also the fact that these pathways differ fundamentally in control, the former being a chemical process, the latter an enzymatic under cellular regulation, should be worthwhile mentioning. I disagree with the distinction between nitrifier-coupled and fertilizer denitrification (fig. 3), since I am not aware of any syntrophic association of nitrite oxidizers and dissimilatory nitrate reducers. In general, this chapter is awfully method oriented, omitting some central questions: how important is "nitrifier denitrification" in soils for N2O emissions, given the compelling evidence that high soil N2O emissions are dominated by canonical denitrification? Secondly, how does nitrifier denitrification differ functionally from canonical denitrification with respect to involved enzymes (e.g. the apparent lack of nos-homologues), external factors, cellular regulation, biochemical function and so on. Hence, I would wish the text was more tuned towards the ecological role of this pathway (e.g. by referring to the possibility of transient NO2- accumulation in soils, coupling to NOB functioning, etc.) and less heavy on methodological details, which, after all, are given in the literature.

Emerging insights 3 – N2O consumption

This chapter choses net consumption of atmospheric N2O ("soil N2O sink") as a point of departure, which seems beside the point, as the ecological relevance of a terrestrial

net N2O sink is controversial and probably constrained to environments poor in electron acceptors. Instead, this chapter should plea for a better understanding of N2O reduction in general, as it is the only process returning reactive N to the atmosphere in a benign form (apart from Anammox, which is not mentioned all). Hence, "understanding of microbial and physicochemical controls on N2O consumption" (634, L. 14) on all "routes" (633, L. 8) should have high priority. However, the focus should be first and foremost on our understanding of denitrification stoichiometry as a pivotal tool for attenuating the net-release of N2O from soils and not on the implementation of an elusive soil N2O sink function into biogeochemical models. Geo-engineering of soils by inoculation of diazotrophs overexpressing nos is a curiosum, which neglects the unsurmountable challenges associated with understanding the survival of inoculates in soils. This chapter provides a valid key-question, but in a wrong context.

Emerging insights 4 – Denitrification

Denitrification has been studied for more than 100 years, which makes it difficult to understand why denitrification should be the "most poorly understood process in the N cycle" (634, L. 20). Again, this confusion owes to the lack of heuristic discipline pertaining to this manuscript. What this chapter probably wants to communicate, is the well-known fact that denitrification is the most difficult to quantify N-cycling process in situ. I fully agree that this has hampered our understanding of N removal on a landscape scale, and should be therefore prioritized. At the same time, I am somewhat critical to advocating "soil-core based gas recirculation systems" (635, L. 28) as a universal solution to the problem. Replacing N2 by He/O2 may be feasible in porous, organic top soils of forests or wetlands, but leads to major artefacts in soil O2 distribution in more densely packed (mineral) soil, when He/O2 has to be flushed through the soil or N2 is exchanged by repeated vaccum/purging with He/O2, thereby effectively oxygenating anaerobic microsites.

Of course, there has been quite some progress in understanding denitrification on a landscape level other than based on estimating in situ rates. Structure-function studies have revealed a sizable diversity of denitrifying phenotypes among indigenous denitrifying communities, which point at adaptation to prevailing environmental conditions with consequences for their biogeochemical functioning. This should be kept in mind when studying "hot spots" and "hot moments" in situ, as these are mainly representations of the organisms' physiologies, controlled by their denitrification regulatory phenotypes. Experiments incorporating "new ideas about hotspots and hot moments" (637, L. 12) should incorporate such findings and guide hypothesis-driven approaches transgressing the usual "black-box" concepts based on well-known, more or less proximal drivers of denitrification.

Finally, what are the "powerfull new tools for extrapolation and validation at regional and continental scales" (637, L. 13)? Soil core studies in He/O2 atmosphere with oxygen based transfer functions? There would be much to say about the shortcomings of this approach in hydrologically connected landscapes. If focusing on landscape,hydrology should come in.

In summary, chapter 2.4 is quite general, and thus falls short to justify the choice of characterizing hotspots and hot moments as a "key-challenge" in denitrification research.

Proximal controllers 1 – soil fauna

This chapter is nicely written, but I am missing a summary paragraph telling to what end we have to understand soil fauna in soil N research. Obviously, there are some endpoints (net-N mineralization, N2O) that are more susceptible to faunal impact than others. Would be nice to get some educated ranking here. Where, in the N cycle, is research on faunal involvement particularly pressing? Modelling the effects of soil fauna on N dynamics (641, L.4) is no research goal on its own right. What for do we want to use the model?

Proximal controllers 2 – plants

This chapter rises an interesting question: does plant species dependent quality of root deposits exert a direct effect on N transformations (641, L. 20 ff. and 642, L. 23 ff.)? It is easy to understand that seasonal changes in root exudation coupled to phenology affect rhizopshere microbial communities, but I find it difficult to retrieve good experimental evidence that plant species composition affects N-cycling on a functional level, other than due to obvious differences in root architecture or occurrence of legumes. For instance, the experiments of Mooshammer et al (2014) suggest that the chemical composition of rhizodeposit should affect microbial functioning, but can this ever be proven in nature? Accordingly, the text writes about "presumed relationships between N cycling parameters" (643, L. 13) and "lack of clear cut relationships" (643, L 23), correctly illustrating the problem. Does this mean that future research on rhizosphere effects should concentrate on broad-scale functional aspects of root architecture and others rather than subtle differences in chemical composition of root deposition? An interesting and important question.

Proximal controllers 3 – mycorrhizal Associations

This chapter rises truly fundamental questions, which should be linked to all other topics dealt with in this review, particlary the fact that many of these processes are studied on disrupted soil samples.

Methods - 15N tracing modelling

This chapter sets off with the ambition to show how 15N enrichment techniques have promoted our understanding of N cycle dynamics in soils (648, L. 25). This is somewhat counter-intuitive as pool dilution approaches do not really cover N cycle dynamics over time (notwithstanding the fact that they emply 1st order kinetics in their numerical solutions), but rather give a snap shot of gross rates in soil. Apart from the discovery of substantial N-turnover in old growth forest soils, the value of 15N enrichment techniques seems to exhaust itself in demonstrating the significance of "heterotrophic nitrification" in forest and grassland soils. This topic has been around for a long time, is reproduced by numerous 15N labelling experiments, but is intimately coupled to the use of numerical models. Therefore, its ecological relevance seems still somewhat dubious. For instance, recent experiments combining numerical modelling of pool dilution and inhibitors could not confirm a universal role of heterotrophic nitrification in two grassland soils differing in pH (e.g. Wang et al., 2014, SBB). What experiments, other than or in combination with numerically solved 15N enrichment pool dillution would be needed to cast light on this long-standing issue? Otherwise, I fully agree that nitrite dynamics should be central to our understanding of N2O emission, the main difficulty being to extract and reliably determine 15N in small NO2- pools.

Specific comments:

624, L. 6: “mitigation of the soil N cycle”. We do not want to mitigate the soil N cycle, do we?

625, L. 20: “Since the 1960s, . . .” Give original literature

626, L.1: What do you mean by “size” of an N-cycling process?

626, L.3-10: I support the focus on N-cycling rates. This is not to say, however, that exploring the microbial genetic makeup in soils and its link to prevailing environmental conditions is futile. Metagenomic approaches, in particular, have been advocated to address multiple biochemical pathways involved in N cycling and to elucidate the role of microbial community dynamics. What is the authors’ opinion on that? Would metagenomics of the soil N cycle contribute significantly to a “research agenda with respect to the N cycle for the next decade” (625, L.1-2)?

626, L.5: Why and how has the molecular revolution in soil science hindered our effort to quantify process rates?

626, L.11 ff: This plea for “soil N cycling process rates” (sensu in situ?) is somewhat single-edged: missing N in mass balances is not necessarily explained by more information on process rates. Often we poke in the dark with respect to which processes

dominate N assimilation or dissimilation in a given ecosystem, i.e. we are lacking information about the nature of the prevailing N transforming processes. Prominent examples are BNF, ammonia oxidation, nitrite oxidation and chemo-denitrification in acid soils. Most severely, we lack knowledge about the partitioning between chemical and biological processes in N dissimilation (nitrosation, ferrous wheel, feammox, etc.). Therefore, rigorous delineation between biotic and abiotic processes is needed in a research agenda to come. Finally, we can hardly advance our knowledge on the soil N cycle without looking at the ecology of the organisms involved, their ecological niches, physiologies, nutrient controls and responses to environmental factors. Closing mass balances cannot be the primary goal and should be tuned down. This paragraph has room for improvement.

631, L. 18: “monoculture studies”; do you mean “pure culture” studies?

634, L.13-15: molecular tools (primers) in denitrification research are heavily biased towards gram-negative denitrifiers, not gram-positive ones!

634, L.14: “Assessment of novel gene expressions”. What is a novel gene expression, rephrase.

642, L21: the taxonomic diversity of denitrifiers is immense, compared to that of nitrifiers.

650, L. 11 ff. As to the use of oxygen labelling, section 2.2 clearly identified limitations of this approach, which should be mentioned also here.

676, figure caption: replace “tropic” by trophic

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