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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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[We have uploaded the revised manuscript separately in our response to the editor]

We would like to thank the reviewer for his/her thorough comments, which really helped to improve the coherence, structure and quality of our manuscript. Below, we respond to all points raised by the reviewer. In order to ensure a complete rebuttal, we have not deleted any text from the original review. Per issue raised, we have clearly indicated the comments of the reviewer as well as our response. In the (relatively few) cases where we disagreed, we have clearly explained ourselves.

Reviewer #1: 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

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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 mycorrhizal 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 C298 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 ecology, 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.

Response: We agree with the comment raised by reviewer #1; similar comments were brought forward by reviewer #2 and #3. Therefore, we have re-classified the topics of this paper in 3 sections. A first section focuses on three basic processes involved in the formation of gaseous N forms: nitrifier denitrification, N2O reduction, and denitrification. The second section focuses on methodological advances in 15N tracing models to elucidate and quantify these pathways of gaseous N production. Finally, the third section describes ecological interactions among soil microorganisms (biological N fixation, mycorrhiza), plants and soil fauna that influence soil N cycling rates. The

SOIL
1, C633–C646, 2015

choice of these topics is now better justified in the introduction of the paper; comes back in the conclusion section; and provides a more coherent conceptual framework to advance our understanding of soil N cycling. We have also adapted Fig. 1 to reflect this restructuring.

Reviewer #1: 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.

Response: We have changed the formulation now. Both pathways are elusive (not illusive, fortunately), but for N2O reduction we know it is very important - it is the final step of denitrification, after all. For nitrifier denitrification we specified now that it is potentially important but that problems associated with its measurement have until now hindered our understanding of this process.

Reviewer #1: 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.

SOIL

1, C633–C646, 2015

Response: Thank you for your kind words on this section. With respect to your remark: The focus of this paper is rather to highlight areas of knowledge gaps rather than focusing on methodological constraints. Hence we decided not to include a section on the 1) methods to assess free-living N2 fixation (ARA vs. 15-N2) and 2) manipulation experiments (focus on N and P and clever selection of niches). This would exceed the scope (and appropriate length) of our manuscript. There are other, more specialized, articles focusing on these issues.

Reviewer #1: 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.

Response: We have clarified the description of nitrification-coupled denitrification. Indeed, it is not a separate process, but it is a pathway which is relevant in many soils, and distinct from "fertilizer denitrification" as the origin of the N is ammonium. The reason that we mention it here specifically is that (as we state in the text) this term has often been used in the past to describe nitrifier denitrification. The main reason to make a clear distinction between Fertilizer denitrification, nitrifier-coupled denitrification and nitrifier denitrification is therefore to be absolutely clear about the terminology. In our experience, this is still necessary. We specified more clearly the nature of NCD in the revised text. We also specified in the revised text that N2O formation during NN is chemical process whereas ND is biochemical, following the suggestion of the reviewer.

Reviewer #1: 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

SOIL
1, C633–C646, 2015

denitrification?

Response: We agree with the reviewer that "how important is nitrifier denitrification" is one of the central questions to be asked. In fact, it is one of the key challenges of our manuscript. However, as opposed to some of the other key challenges that we ask, the main constraint in answering this question here is one of methodology. We simply cannot say how important it is, as almost no studies yet have been published that quantify it accurately. The main purpose of this section is therefore one of terminology and methodology. We have tried to explain more clearly the different nature of the key questions which we formulate in our manuscript.

Reviewer #1: 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.

Response: We have added two paragraphs on the nature of nitrifier denitrification; its enzymatic relation with (but functional difference to) canonical denitrification as well as the difference with nitrifier nitrification.

Reviewer #1: 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)

SOIL
1, C633–C646, 2015

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.

Response: In the revised manuscript, we have better described the context for N2O consumption in soil and rearranged for that purpose the first paragraph of section 2.2. We focus the section now more on the need to understanding the role of N2O reduction for attenuation of the net soil N2O release. Nevertheless, if the latter is relevant, a next logical step is the inclusion of in situ N2O consumption in biogeochemical models. We did not really argue for geo-engineering of soils; the latter was just used as an experimental tool to demonstrate in situ N2O consumption. We actually do refer to Anammox in Figure 3. However, we believe that the concepts of this process or relatively well understood, and that its importance for the soil N cycle is likely to be limited. For this reason, we did not included it as one of the key points of this manuscript. We changed the formulation on page 634 of the original manuscript. It now reads: “Hence, we propose that the expression of novel, recently discovered genes involved in N2O consumption in conjunction with the quantification of N2O fluxes in various soil types is required to advance our understanding of microbial and physicochemical controls on N2O consumption, and ultimately to develop improved biogeochemical models of soil N2O sink function.”

Reviewer #1: 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

SOIL
1, C633–C646, 2015

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.

Response: We have removed the assertion that denitrification is the "most poorly understood process in the N cycle." The text now focuses more clearly on the fact that denitrification is difficult to measure.

Reviewer #1: 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.

Response: We have revised the text to clarify that the soil-core based gas recirculation systems are certainly not the final answer to the challenge of measuring denitrification. We have revised the first sentence to say that "our understanding of the N2 flux associated with denitrification has been improved at least somewhat by the development of soil core-based gas recirculation systems" and there are two sentences at the end of this paragraph highlighting the drawbacks of this method.

Reviewer #1: 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

SOIL

1, C633–C646, 2015

approaches transgressing the usual “black-box” concepts based on well-known, more or less proximal drivers of denitrification.

Response: We have added a short paragraph to make the important point that “Experiments incorporating new ideas about hotspots and hot moments can benefit from recent studies that have characterized diversity in denitrifying phenotypes that reflect adaptation to prevailing environmental conditions with consequences for denitrification activity (Bergaust et al. 2011). These ideas have the potential to improve these experiments by allowing for more mechanistic, hypothesis-driven approaches that underlie more “black-box” ideas based on proximal drivers of denitrification.

Reviewer #1: 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.

Response: We have revised this paragraph to have it more effectively summarize this section of the paper and eliminated references to extrapolation to regional and continental scales that we do not address.

Reviewer #1: 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.

Response: We hope that the section is now more specific and that it does a better job of justifying the importance of hotspots and hot moments of denitrification as an exciting area in soil research.

Reviewer #1: 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,

SOIL

1, C633–C646, 2015

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?

Response: We agree with the reviewer that such a summary paragraph is missing. We have added it at the end of this section, following the suggestion of the reviewer to shortly outline our view on the future focus of research in this field.

Reviewer #1: 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.

Response: Reviewer #1 seems to agree with our general vision on links between plant traits and soil N cycling. We indeed believe that both the biochemical composition as well as root architectural traits deserve further attention.

Reviewer #1: Proximal controllers 3 – mycorrhizal Associations. This chapter rises truly fundamental questions, which should be linked to all other topics dealt with in this

SOIL
1, C633–C646, 2015

review, particularly the fact that many of these processes are studied on disrupted soil samples.

Response: The fact that many studies that assess soil N cycling rates have been performed on disrupted soil samples is explicitly mentioned in the section on rhizodeposition and plant traits. We agree with the reviewer that this issue is not limited to mycorrhizal communities as disturbing soil sampling prior to soil N cycling assessments impacts on the entire soil microbial community, including free-living and symbiotic communities.

Reviewer #1: 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, C303is reproduced by numerous 15N labelling experiments, but is intimately coupled to the use of numerical models. Therefore, its ecological relevance seems still somewhat dubious.

Response: We do not fully agree with this comment. First, the cited study on old-growth forest was only the first to show substantial gross N turnover despite low net rates, which since has been confirmed many times. In addition to this, 15N labelling techniques have, as discussed, demonstrated the importance of heterotrophic nitrification (even though long around, still debated widely) and of DNRA. This has demonstrated more complex N2O production dynamics and the significance of depolymerization of the N cycle.

For instance, recent experiments combining numerical modelling of pool dilution and

SOIL

1, C633–C646, 2015

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?

Response: We do not claim that heterotropic nitrification is universally important in all grassland and forest soil, but rather that it “can be a significant or even dominant” pathway. For clarity we rephrased this statement somewhat in the revised manuscript: “. . ., but contrasting results exists”. Furthermore, we added additional text and a reference on the need for better understanding controlling factors for heterotrophic nitrification. 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. Response: Thank you.

Reviewer #1: Specific comments: 624, L. 6: “mitigation of the soil N cycle”. We do not want to mitigate the soil N cycle, do we?

Response: We rephrased this, it now reads "...understanding, measuring and altering the soil N cycle."

Reviewer #1: 625, L. 20: “Since the 1960s, . . .” Give original literature

Response: We do not quite understand this point. The two papers which we cite (Compton et al., 2011; Davidson et al., 2012) are quite recent, but have the benefit of giving an historic overview of man’s relation with the N cycle and are therefore (if anything) better able to describe the general trends we are referring to, and in present day terms than the contemporary literature.

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

Response: We changed this into "flux rates".

Reviewer #1: 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

SOIL

1, C633–C646, 2015

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)?

Response: We agree that investigating the link between genetics and N cycling processes holds promise to advance our understanding of the N cycle. Molecular based approaches, including metagenomics, have the potential to investigate the presence of a large number of genes. Nevertheless, it should be clear that gene presence is a proxy for potential activity; genes may be present even in case the organism is not functionally active in the N cycle. Therefore, we believe that microbial molecular techniques should always be used in combination with process based measurements. We have indicated this in the revised Conclusions section.

Reviewer #1: 626, L.5: Why and how has the molecular revolution in soil science hindered our effort to quantify process rates?

Response: This section has now been changed and this statement has been removed.

Reviewer #1: 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

SOIL
1, C633–C646, 2015

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.

Response: We agree with the reviewer. In fact, our whole manuscript is a plea for a concerted effort to get more insight in the soil N cycle by a combination of studying the nature of processes; their influence by ecology; as well as quantification of transformation rates. The original paragraph suggested that we would focus mostly on the latter. We have now restructured the whole section to better reflect the character of our manuscript. We do not really see the special importance of distinguishing between biotic and abiotic processes - we selected the key challenges to be studied without a priori excluding chemical processes.

Reviewer #1: 631, L. 18: “monoculture studies”; do you mean “pure culture” studies?

Response: Yes, we changed it. Thank you!

Reviewer #1: 634, L.13-15: molecular tools (primers) in denitrification research are heavily biased towards gram-negative denitrifiers, not gram-positive ones!

Response: We changed this, thank you.

Reviewer #1: 634, L.14: “Assessment of novel gene expressions”. What is a novel gene expression, rephrase.

Response: We rephrased the whole paragraph.

Reviewer #1: 642, L21: the taxonomic diversity of denitrifiers is immense, compared to that of nitrifiers.

Response: We agree that the taxonomic diversity of denitrifiers is much wider compared to nitrifiers. Nevertheless, if compared to the mineralization process, both nitrate producing and consuming processes can be considered as phylogenetically narrow. However, we adapted the text. It now reads "Nevertheless, nutrient availability in

SOIL
1, C633–C646, 2015

the rhizosphere and competitive interactions between plant and microbial communities may shift the magnitude and direction of N cycling processes. This holds especially true for those processes that are performed by phylogenetically less diverse microbial functional groups; processes such as nitrification and methane uptake should therefore be much more sensitive to shifts than N mineralization (Philippot et al., 2009; Dijkstra et al., 2013)."

Reviewer #1: 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.

Response: We added the following sentence: "The limitations and opportunities of this approach are discussed in Sect. 2.1" (The section numbering has been changed).

Reviewer #1: 676, figure caption: replace "tropic" by trophic

Response: Changed. âĂČ

Interactive comment on SOIL Discuss., 1, 623, 2014.