



Peer Community In Ecology

Nitrate or not nitrate. That is the question

Sébastien Barot  based on peer reviews by **Vincent Maire** and 1 anonymous reviewer

Legay Nicolas, Grassein Fabrice, Arnoldi Cindy, Segura Raphaël, Laîné Philippe, Lavorel Sandra, Clément Jean-Christophe (2020) Studies of NH_4^+ and NO_3^- uptake ability of subalpine plants and resource-use strategy identified by their functional traits. Missing preprint_server, ver. Missing article_version, peer-reviewed and recommended by Peer Community in Ecology. [10.1101/372235](https://doi.org/10.1101/372235)

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The article by Legay et al. [1] addresses two main issues: the links between belowground and aboveground plant traits and the links between plant strategies (as defined by these traits) and the capacity to absorb nitrate and ammonium. I recommend this work because these are important and current issues. The literature on plant traits is extremely rich and the existence of a leaf economic spectrum linked to a gradient between conservative and acquisitive plants is now extremely well established [2-3]. Many teams are now working on belowground traits and possible links with the aboveground gradients [4-5]. It seems indeed that there is a root economic spectrum but this spectrum is apparently less pronounced than the leaf economic spectrum. The existence of links between the two spectrums are still controversial and are likely not universal as suggested by discrepant results and after all a plant could have a conservative strategy aboveground and an acquisitive strategy belowground (or vice-versa) because, indeed, constraints are different belowground and aboveground (for example because in given ecosystem/vegetation type light may be abundant but not water or mineral nutrients). The various results obtained also suggest that we do not full understand the diversity of belowground strategies, what is at stake with these strategies, and the links with root characteristics. Each time I give a conference on the work we are carrying out on African grasses that likely absorb ammonium preferentially because they inhibit nitrification [6-7], somebody asks me a question about the fact that plant essentially absorb nitrate because ammonium is toxic and nitrate more available in the soil. The present article confirms that this is not the case and that, though there are currently some teams working on the subject, we do not really know for the moment whether plants absorb nitrate or ammonium, in which proportion, how plastic this proportion is within individuals and within species. This subject seems to me crucial because it is linked to (1) the capacity of ecosystems to conserve nitrogen [8], because nitrate, much more than ammonium, goes out of ecosystems through leaching and denitrification, (2) to carbon cycling and plant energy budget because absorbing nitrate requires spending mucho more energy than absorbing ammonium because nitrate

must be reduced before being incorporated in plant biomass, which is very energy costly. These two issues are naturally very relevant to develop efficient cropping systems in terms of carbon and nitrogen. Interestingly, the present article, comparing three grass species in different sites, suggests that there is no trade-off between the absorption of nitrate and ammonium: more acquisitive individuals tend to absorb more ammonium and nitrate. This is contrary to hypotheses we made to predict the outcome of competition between plants absorbing nitrate and ammonium in different proportions [9] but should be tested in the future comparing many different types of plants. The results also suggest that more conservative plants absorb relatively more ammonium, which makes sense because this allows them to spare the energy necessary to reduce nitrate. This leads to the question of the effect of these strategies on nitrogen retention within the ecosystem. If nitrification is high (low), absorbing ammonium is not efficient and likely leads to high (low) nitrogen losses. This should be tested in the future. Moreover, the authors have measured the absorption of nitrate and ammonium through measurements at the root scale on cut roots. This should be complemented by measurements at the whole plant scale.

References:

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Reviews

Evaluation round #2

DOI or URL of the preprint: <https://doi.org/10.1101/372235>

Version of the preprint: V2

Authors' reply, 20 December 2019

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Decision by Sébastien Barot , posted 15 December 2019

Revision required

I am satisfied with the way the manuscript has been improved and the reviewers' comments addressed. I would be ready to recommend the manuscript but have a few more comments.

The abstract could mention the fact that the preference for ammonium vs. nitrate is different between species and higher for conservative species.

I like the discussion however:

- 1) It should explicitly mention the fact that the interpretation is limited by the fact that only three species are studied.
- 2) I think the way nitrate and ammonium uptake rates are measured is very useful but I think the discussion/conclusion should mention that it would also be interesting to measure the absorption of nitrate and ammonium at the whole plant scale. For example, through ¹⁵N pulses. This could give a different image of N uptake.
- 3) I like the discussion about the differences between the relative nitrate and ammonium rates and the link with the plant strategy. However, I am surprised it is never mentioned that assimilating ammonium is less costly than assimilating nitrate (that has to be reduced). This could be in line with a more conservative strategy? Similarly the influence, of the ammonium vs. nitrate preference likely has consequences on ecosystem functioning and the N budget of the ecosystem (because nitrate is more prone to losses) (see Boudsocq 2009 and 2012, OK I am co-author of these articles).
- 4) Ammonium is more absorbed during autumn. Could that just be due to the fact that ammonium is less mobile than nitrate within the soil so that it requires more humid soils to be absorbed?

There are still some writing glitches. I have listed some of them (see below) but the manuscript should be carefully proofread.

Line 55. It is awkward to start the sentence with "And". I think the "plant ecology," should be deleted

Line 60. I suggest "the significance of root traits is less understood than the one of leaf traits"

Line 66. "is both influenced by anatomical and by physiological adjustments such as ..."

Line 69. "Nitrogen is one of the best studied mineral nutrients and its uptake by plants under both the ammonium and nitrate forms is influential for plant and ecosystem functioning"

Line 72 "some information" à la place de "supports"

Line 100. Could the first hypothesis be expressed more precisely? What does that mean "contributing to the economic spectrum"? Does that mean that there is a root economic spectrum fully correlated (positively) to the leaf spectrum?

Line 105. I do not understand the "both in quantity and quality"

Line 130. "managements"

Line 146. "during the day"

Line 151 "kept in ice"?

Line 152 "5.6 mm mesh"
Line 164 "living young roots"
Line 174 "root 15N natural"
Line 183 "at the cost of losing relevant ecological information"
Line 210 "Hanes's relations were used" or "Hanes's relation was used"
Line 269 I do not understand this sentence because obviously the paragraph is comparing different sites
Line 287 I find that "resource use " is too vague and not related enough to the previous sentence
Line 288 "despite relatively weak relationships"
Line 289 "different selective pressures" "specializations"
Line 304 "indeed" seems to me inappropriate here
Line 326 "opposite response" is for me too vague. Response to what? Response of what?
Line 359 "could depend on"
Line 364 The sentence is in my opinion awkward. Should the plural be used? Not clear whether this is a result of the manuscript or a general thought coming from the reference.
Line 379 "remain"
Line 397 "grassland N cycling rate"

Evaluation round #1

DOI or URL of the preprint: <https://doi.org/10.1101/372235>

Authors' reply, 12 December 2019

[Download author's reply](#)

Decision by Sébastien Barot , posted 16 October 2018

Revision required

Dear authors Your manuscript is interesting. However, the two reviews are pointing at potential problems that should be addressed before any final decision is made. I am looking forward reading a new version of the manuscript together with answers to the reviewers. I apologize for delay in sending my decision. It has indeed been difficult to find reviewers during the summer period.

Reviewed by Vincent Maire, 19 September 2018

[Download the review](#)

Reviewed by anonymous reviewer 1, 14 August 2018

The ms compared for three grasses in four different grasslands (but only one grassland contained all three species!) patterns of ammonium and nitrate uptake capacity in relation to several aboveground and belowground functional traits. When reading the ms I noted that very little literature post-2010 was cited. I found this curious, as the field of root traits (and their possible coordination with leaf traits) is a flourishing research field. However, the explanation is simple: the ms is just around four years old; and rather than let it rest in peace the authors decided to submit it. However, they cited a paper by Grassein et al. in press (l. 467; note that in the text, but not in the references there are papers by Grassein et al. 2010, 2015 & in press). But the paper in press (in Ann. Bot.) was published in January 2015 (with on-line availability at the end of 2014). As I do not think it is the duty of reviewers to come up with suggestions of important literature, when the authors could easily have done so themselves, I will not go in details where the paper could be improved. The paper is

also sloppy in other respects. The description on functional traits is partly repetitive (SRL is mentioned twice), while the paper makes inconsistent claims how long excised roots were stored before measurements (l. 161 mentions less than 2 hours, l. 177 less than sixty minutes). Again, these mistakes should have been seen by at least one of the six authors! I will only give a few examples where I think that more careful ecological considerations would have been important.

The authors decided to measure V_{max} rather than K_m (although the text in l. 211 suggests that the latter parameter was also measured but not reported). However, they did not provide a rationale for it. As they studied the high-affinity uptake systems they looked at uptake at low concentrations. Under such conditions plants may be selected to optimise K_m to ensure a sufficient influx of nutrients under conditions of low availability. While V_{max} is potentially important (especially in cases of clear nutrient pulses, e.g. immediately after snow melt or after sufficient moisture at the end of the summer), I think the authors should have presented both parameters and should give a rationale why maximum uptake capacity rather than K_m would fit better with a plant economics spectrum.

The authors also report to have measured $\delta^{15}N$ at natural abundance (l. 169) but do not report them. While there has been substantial debate to what extent these data relate to differential uptake from separate sources (peoples have overconfidently used such data to link these results to mycorrhizal preferences for various N sources) and / or to isotopic discrimination during uptake, I think it would have been helpful if the authors has measured the ^{15}N signature of both ammonium and nitrate in their soils. Such data could also increase the ecological significance. The authors decided not to supply both nutrients at the same time, but I did not read a rationale why this would have been important. Considering that N-uptake has a major effect on the proton balance in the rhizosphere, may plants would likely take up both with a mixed supply to minimise pH-changes in the rhizosphere. In terms of functionality, such a mixed uptake system may be very relevant. A view that looks at these as independent (and synergistic –l. 298; how was this tested?), misses functionality of uptake systems.

The design is pretty minimalistic (three grass species; one grassland in which all three species occurred; and three grasslands where only one species was found). And disentangling species effects from site effects is only possible with $n = 2$. In order to increase significance of their data, the others sampled more plants per site. At a distance of a few meters, one could differ in the opinion whether these constitute 'true' replicates or pseudoreplicates (which increase the degrees of freedom and hence the significance of statistical tests; only Fig. 2 seems to aggregate the individual root samples into one average value with 12 data points). The other sampled during two seasons and described the roots sampled (l. 160) as young fine roots. There is little detail and one wonders whether root age and root nutritional status (rather than seasonality) contributed to seasonal differences. (There could also have been differences in mycorrhizal colonisation in both seasons, and even though these three species show only limited growth responses to mycorrhiza, it does not follow that differences in uptake patterns are related to difference in mycorrhizal fungal mycelium surrounding the roots).