

**Dear managing editor,  
Dear authors,**

**The 19<sup>th</sup> of October 2018**

The manuscript, entitled “Studies of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> uptake ability of subalpine plants and resource-use strategy identified by their functional traits” by Fabrice Grassein and coauthors proposes in a research paper to study the intraspecific variability of leaf and root functional traits related to plant nitrogen nutrition along an intensity management gradient and throughout two seasons for three grass species. The objective of the paper is to study how root functional traits are related with the leaf economic spectrum and to test if the root nutrition strategy for nitrogen (maximal uptake of N, preference for NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup>) is related with the leaf N utilisation strategy as defined according to the leaf economic spectrum. This manuscript is written with a good English, and results are of interest for the readership of PCI Ecology. The study will be particularly relevant in plant functional ecology and grassland ecology disciplines.

I found that the study suffers from two main major issues that prevent to give my support for acceptance. Let me explain: All the introduction, theoretical and statistical approaches as well as objectives and discussion of the study are presented to focus on differences between species. However, the study design and sampled data (including the number of species which is three) are mainly based to test the differences within species. There is then a discrepancy between what the authors want to test and their message and what the data permit to do. For instance, the leaf economic spectrum or the NO<sub>3</sub><sup>-</sup> / NH<sub>4</sub><sup>+</sup> trade-off have been observed at the interspecific level. However, you cannot expect to address interspecific differences with three species. In addition, it is not really clear if interspecific patterns occur at the intraspecific level too. It seems that correlations between traits involved in the leaf economic spectrum are weaker or even broken at intraspecific levels (Messier et al 2010 ELE; Derroire et al 2018 Scientific Reports; Fajardo and Siefert 2018 Ecology; Osnas et al 2018 PNAS). At least, one would expect to show how the trait variations of this study fit with the worldwide or grassland LES or with the NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> trade-off as the data are easily available in the literature or through TRY database. Then, I strongly suggest to the authors to better streamline the conceptual framework on which their interesting results will be interpreted. I do not think that LES or NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> are the good ones. I suggest to better focus on the intraspecific variation across the season and along the management gradient.

The second important issue concerns how authors define the nitrogen uptake term, which is key in their study but has not been clearly defined. All over the article, we have the feeling that nitrogen uptake is the total nitrogen that plant acquire along the season. However, this is the root instantaneous maximal uptake rate of either nitrate or ammonium and the leaf N content that have been measured and used in results. By the way, the root instantaneous maximal uptake rate of total mineral nitrogen does not appear in the results. Altogether, this leads then to many confusions when authors discuss their results (for instance L336-337). It will be very important to clearly define what plant N uptake is to further understand the results and their interpretation.

Other important issues but less problematic to me are listed below:

## **Abstract**

The abstract is too general and do not give enough result to understand what have been done. How many species, many grasslands, and seasons did you investigate for instance? L29 What is the plant resource economic spectrum RES? It is defined neither in the abstract nor in the introduction.

## **Introduction**

L11-12 The leaf economic spectrum seems to not have been fully understood. First, some important traits of the LES were not measured for this study but are central to the LES: leaf photosynthetic capacity and leaf lifespan for instance. Second, high nitrogen use efficiency belong more to the conservative strategy rather than the exploitative one. Third, photosynthetic capacity and photosynthetic N use efficiency are two different variables with different meaning but are presented as the same variable (L49).

L50: Reich et al 1998 is lacking in the reference list.

L6-74 One would consider that some N transporters are constitutive while others are adaptive. As such, it is important to study interspecific differences for N, Nitrate and Ammonium at a given site, which is not the case in the study. Otherwise, it is difficult to interpret differences as resulting from species differences. Please mention this point.

L83-L92 This paragraph needs to be rewritten. First, it is not clear why a methodological point is introduced in the introduction. It seems to me that it is not important to present this here. Second, this paragraph is very unclear suffering from a bad logical flaw and very long sentences.

L94 What do you mean by ‘contrasted LES’. There is only one universal leaf economic spectrum. Do you mean contrasted leaf economic strategies?

L101 It seems that the objective 2 has never been statistically tested. I do not see any result where the total nitrogen uptake is analysed.

L103. The number 3 of the third hypothesis seems to be wrongly located in the sentence. I would place it before ‘As functional traits...’

## **Method**

L109: Please quote table 1.

L161 and L192 - CaSO<sub>4</sub>: It seems problematic to me to use the same solution in order to preserve the integrity of roots before uptake measurement as well as to stop the uptake of nitrate and ammonium at the end of the root incubation. Please clarify this.

Data analysis is another major issue of the article! First, it is totally obscure how many data are used for each analysis. For instance, one would expect to see 15 points (3 species \* 5 individuals) or 24 points (4 habitats \* 2 seasons \* 3 species) on figure 2, while only 12 are presented without any explanation. Second, the statistical models used to test differences are not appropriate with the hierarchical (site, season, species, individuals) and unbalanced design of the data (not the same number of species for the different sites).

L211: Km, which seems to be N affinity, has not been defined. Please remove it as you do not use it in the study. Finally, it is not clear how each statistical tests address each of the three hypotheses of the introduction. Please give more details.

## **Result**

L234: Looking at table 3, LNC seems also a good predictor of nitrogen uptake.

Figure 2: Please use subscript and superscript in the y axis legend. Please use only two digits for root square

### **Discussion**

L270-273 You use three negatives in the same sentence, resulting a very unclear sentence. Please remove at least two negatives.

L274 It is unclear why ‘similar’ refers to. Please clarify it.

L283-285 The statistics used here are not appropriate to test the causality and it is then not appropriate to say that leaf traits determine root traits. In addition, other studies show that root N maximal uptake rate is strongly correlated with leaf nitrogen content (Osone et al 2005 ABO; Soussana et al 2005 NPH; Osone et al 2008 NPH; Maire et al 2009 FEC), which is in contradiction with the assertion proposed in this sentence.

L305-306 It seems very contradictory with the sentence 283-285. Please clarify this.

L322 TDN has been defined far away from this place. Please write total dissolved nitrogen

L336-338 ‘the effects of soil NH<sub>4</sub><sup>+</sup> : NO<sub>3</sub><sup>-</sup> ratio concentration on plant N uptake’ are not strictly presented in your results. Please do it if you want to assert some purpose.

L391-395 Only one year has been investigated. It is then not possible to conclude on a seasonal effect. Multiple years of investigation would be necessary for that.

I read your paper with great interest and I believe it is very relevant to PCI Ecology readership, providing the consideration of the issues presented here.

Hope will the comments be useful.