**Biogeosciences
Discussions**

Interactive
Comment

Interactive comment on "Diversity pattern of nitrogen fixing microbes in nodules of <i>Trifolium arvense</i> (L.) at different initial stages of ecosystem development" *by* S. Schulz et al.

S. Schulz et al.

stefanie.schulz@helmholtz-muenchen.de

Received and published: 14 December 2012

General comments: This manuscript addresses questions of great relevance for ecosystems under development. Although it does not answer all questions related to the potential of nitrogen fixation in these ecosystems, it certainly represents a starting point. The conclusions are to a certain extent valid, but I would argue that they would have had much more weight if, for instance, more samples had been analyzed. From a statistical point of view you are right, more samples might reduce standard deviations and therefore increases robustness of results. Therefore a good compromise between the statistical view, the aim of a study and the handling of samples and data

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

is essential. In our case we wanted to get a first inside in the symbiotic nitrogen fixation process/establishment at the beginning of ecosystem development and as you already mentioned this is a good starting point. We agree that for future studies it might be worth to zoom in to a specific question with a higher resolution (see also future perspectives). Anyhow, to confirm the reliability of the results we want to show one example: We analysed the distribution of the nodules for 90 plants in total (Figure 1). As the result was the same as for the 18 plants (Figure 2) we used for further analyses, we decided to show all data related to the analysed plants only.

I have a few general remarks that should be taken into account when discussing the results.

First, it refers to the fact that the authors collected samples from a developmental stage where clover had already exceeded the highest density. Wouldn't be interesting to take samples from sites where highest density is found? In principal it was our aim to compare a site where clover recently appeared and one where clover was already established for a couple of years. Although, the highest density of Trifolium was reached in the year before, Trifolium still remained the dominant plant species at the site. We changed the sentence to point out the clear difference between Trifolium coverage: "…where T. arvense recently appeared (density below 5%), and a site 5 year (5a) after initial ecosystem development started, where the clover revealed a density of up to 25%."

In the same lines, I miss description about each developmental site in terms of plants species, specially other legume plants, as this could influence the relative abundance of nifH harboring bacteria in the soil. We included a more detailed description about plant coverage and dominant plant species in the Material and Methods section: "The sampling site in the east of the Chicken Creek was constructed as part of the catchment and has thus the same development stage. Plant coverage was between 25 and 35%. The dominant plant species were Trifolium arvense, Calamagrostis epigejos, Echium vulgare, Daucus carota and Cirsium arvense. The second site in the west

Interactive
Comment

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

of the Chicken Creek was restored to "point zero" in 2008, thus the progress of soil development is three years behind the artificial catchment. Only 5 to 10% of the site was covered with vascular plants. Dominant plant species were Conyza canadensis, Tussilago farfara, Calamagrostis epigejos, Echium vulgare and Trifolium arvense."

My second remark refers to the strong fluctuations in nifH copy numbers found per soil age or nodule size. Specifically, I would suggest performing the same analyses for more nodules per treatment. Indeed we have tried to analyze abundance of nifH in more nodules of one size class respective soil type confirming the large differences described in the manuscript (see also above for nodule size classes). We must conclude that there might be also other factors which could be the drivers for this observation, like plant performance and the supply of nodules with plant derived assimilates. As the major idea of this manuscript was to directly compare nifH gene diversity and abundance in the same nodules, we did not include this information, as including more or even different nodules for the abundance analysis would falsify this correlation.

Third remark is about sampling, which was performed at one moment of the year. I can imagine that the size of the nodules will greatly vary according to the developmental stage of the plant. I advise the authors to add few sentences in the discussion explaining how they would expect the size of the nodules to vary at different stages of plant development. It is difficult to speculate about changes in nodule size for wild legumes. The reviewer is absolutely right, when experiments with inoculated strains are performed, then plant development stage might have the greatest impact on nodule size. But in a system with wild legumes a lot of factors control nodulation, starting with the indigenous rhizobial community being effective in infecting T. arvense or not. However, it is know that the majority of nodules from wild legumes often remain small. Moreover, sampling took place during the flowering stage of T. arvense, which is the peak of nodulation, as most nitrogen is needed during that plant development stage. Therefore, we would assume a reduction of nodule numbers and size after flowering and no significant increase of medium or large nodules anymore. We included some

sentences about changes in nodule size and abundance in the discussion. "It is very likely that the peak of nodule abundance was reached at the time point of sampling, because plants were in the flowering stage where most nitrogen is needed (Malhi et al., 2007). At both sites the majority of nodules was small, which is in line with observation of Zahran et al. (1998), who stated that wild legumes mostly form small nodules. This is attributed to the fact that indigenous rhizobia are often adapted to the harsh environmental conditions but at the same time are less effective in forming the symbiosis (Wielbo et al., 2010). ... Assuming that R. leguminosarum from OTU5 is the most effective ecotype in nodulating T. arvense at this site, the small nodules being composed of R. leguminosarum from OTU5 might have the potential to develop to a medium or large nodule over time." We agree with the reviewer that anyhow the topic of nodule dynamics is of high interest mainly when the size of nodules can be linked to the amount of fixed nitrogen. We therefore included this aspect in the section where future research needs are defined. "This study was based on one sampling time point during the vegetation period. Although we assume that during flowering a maximum of nitrogen is fixed in the nodules, nodule dynamics over time might be indeed a topic of interest for future research, mainly when the size of the nodules can be linked to their particular contribution to nitrogen fixation.

Specific comments: Ln 298: could you specify what Invsimpson measures and why it was chosen? The Invsimpson is an estimator of the samples diversity. It is the inverse (1/D) of the classical Simpson diversity index (D). It is argued that the ecological relevance of this diversity index is higher than that of the Shannon diversity index or others. The formula is:

Invsimpson = 1/D

D = $\sum (ni(ni-1))/(n(n-1))$

where ni is the number of individuals in the i-th OTU and n is the total number of individuals in the community. We included the formulae in the text of the revised version

for clarification:

"The Invsimpson index is the inverse (Invsimpson = 1/D) of the classical Simpson diversity index (D), which is calculated by the formular D = $\sum (ni(ni-1))/(n(n-1))$

,where ni is the number of individuals in the i-th OTU and n is the total number of individuals in the community."

Ln 346: please explain what indeterminate nodules are and their relevance. One can distinguish between determinate and indeterminate nodules. The first are characterized by their round shape, which is caused by the loss of the meristematic activity directly after the initation. Thus growth is only attributed to cell expansion. In contrast, indeterminate nodules are able to grow, by producing new cells, during their whole life span resulting in a rather cylindric shape. These nodules are typical for Trifolium species (Gage, 2004; Sadowsky and Graham, 2006). We included the description of the nodule shape briefly in the result part and added two citations in the discussion section: "In total 297 nodules of an indeterminate shape were collected from 18 plants, 154 from the 2a site and 143 from the 5a site."

Gage, D. J.: Infection and invasion of roots by symbiotic, nitrogen-fixing rhizobia during nodulation of temperate Legumes, Microbiol Mol Biol Rev, 68, 280-300, 2004.

Sadowsky, M., and Graham, P.: Root and stem nodule bacteria of legumes in: The Prokaryotes, edited by: Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., and Stackebrandt, E., Springer Science + Business Media, New York, 818-841, 2006.

Ln 369: according to lines 297-298, site 5a had highest diversity Due to major changes in that part of the discussion this sentence was deleted. "However, obviously the medium sized nodules did not drive the difference between the sites. In contrast to the medium sized nodules, Adonis permutation tests revealed a significant influence of soil age on the nifH harboring microbial community of the small sized nodules. The differences are mainly based on an increase of clones coding for R. leguminosarum

bv. trifolii and in addition to a decrease of species from cluster I."

Technical corrections: ln 58: "To address these questions. . . " Which questions? Replace questions by issues Has been replaced in the revised version.

ln 99: "due to that. . ." due to what? Not clear. The sentence has been rephrased in the revised version: "Due to the higher environmental stress level at the very beginning of ecosystem development,"

Ln 154: replace respectively by and This paragraph was changed to clarify the experimental performance: "For nucleic acid extraction, from three plants per plot a small (< 2 mm) and a medium (2-5 mm) sized nodule was used. The three nodules from the same size class were pooled and DNA was extracted by thermolysis."

Ln292-295: this sentence is too long and very difficult to follow. Please divide in two parts The sentence was shortened and divided in two parts in the revised version: "In the clone libraries from the medium sized nodules and from the small sized nodules from plants grown on the 2a site, sequences clustered in three OTUs each. In contrast, calculations resulted in six OTUs for the sequences of the small nodules from plants grown on the 5a site."

Ln 297-300 and The sentence was rephrased in the revised version: "While highest diversity was estimated for nodules from plants sampled on the 5a site covering 6 OTUs and resulting in an Invsimpson value of 3.59, the sequences where evenly distributed among three OTUs for the small nodules from the 2a site."

ln 360-364: same as above As the focus of the discussion slightly has changed in the revised version, this part was deleted from the discussion.

Figure 3: Please add "of nifH gene sequences" after ". . .clone libraries". Has been added.

Figure 4: please add "partial" before ". . .nifH gene sequences. . ." Has been added.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

Interactive comment on Biogeosciences Discuss., 9, 13135, 2012.
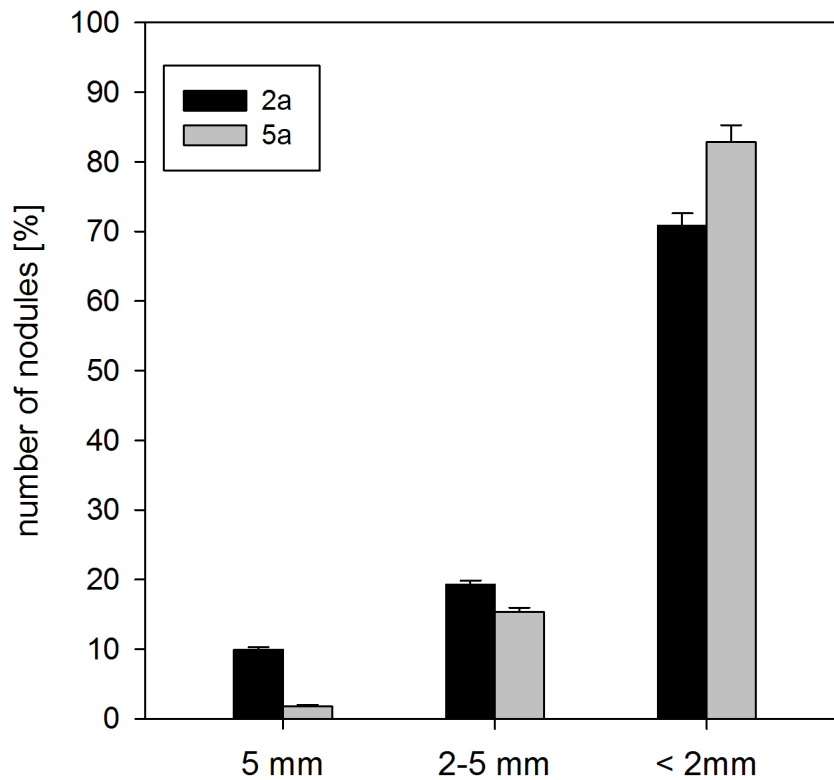
Fig. 1. Distribution of the different nodule size classes, including 30 plants per plot.
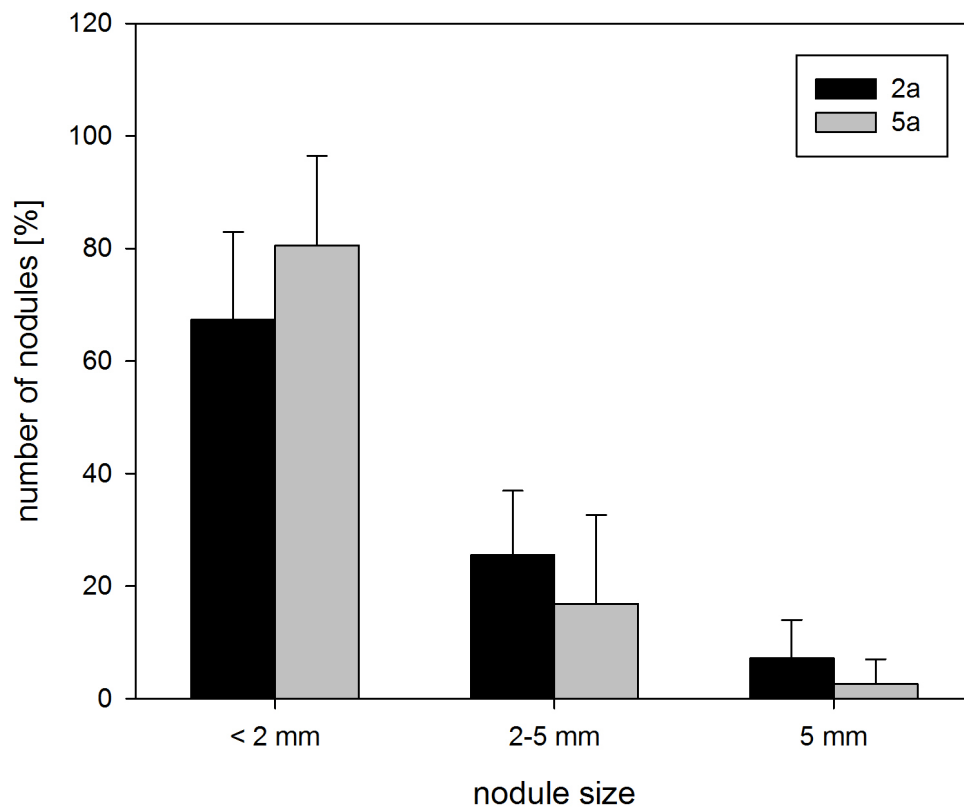
Fig. 2. Distribution of the different nodule size classes, including 9 plants per plot.