

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

Anonymous Referee #1

Received and published: 5 October 2012

Detailed evaluation of the manuscript: Does the paper address relevant scientific questions within the scope of BG? Yes

Does the paper present novel concepts, ideas, tools, or data? Concepts are not new, but work is well-made and provide some interesting data

Are substantial conclusions reached? In most cases – yes

Are the scientific methods and assumptions valid and clearly outlined? Generally - yes

Are the results sufficient to support the interpretations and conclusions? Not at all – my comments and suggestions below

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Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? Yes (definitely!)

Do the authors give proper credit to related work and clearly indicate their own new/original contribution? Yes

Does the title clearly reflect the contents of the paper? Yes

Does the abstract provide a concise and complete summary? Yes

Is the overall presentation well structured and clear? Yes (definitely!)

Is the language fluent and precise? Yes

Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? Yes

Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated? Minor revisions, mostly concerning Discussion section, are suggested

Are the number and quality of references appropriate? Yes

Is the amount and quality of supplementary material appropriate? Yes

Reviewer's suggestions and comments:

Introduction section, p. 4, l. 25-27: “. . .we assumed that the level of diversity of rhizobia nodulating clover will change with ongoing succession, being more diverse at the beginning of ecosystem development.” Why? Please write any justification for this assumption. Rhizobium-legume symbioses are very specific, and, for example, *R. leguminosarum* bv. *trifolii* will not nodulate alfalfa, regardless of presence or absence of *S. meliloti* in soil (no compatible microsymbiont = no symbiosis). Of course some non-rhizobial bacteria could be sometimes isolated from nodules, but they are rather additions, “contaminations” which are present in nodules together with rhizobia, but not

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real nitrogen-fixing microsymbionts which are able to induce nodulation.

Results section, p. 10 l.3-18 It should be clearly stated which differences are statistically significant, and which are not. This was written for differences in C/N ratio (p.10, l. 16-17) but I think this should be done also for other values reported in this part of the manuscript.

Results section, p. 11, l.13-14: "The maximum number of OTUs per nodule was 3". Is it true? In Material and Methods section (p.6, l. 23-24) Authors wrote: "Plants from three different plots were treated as true replicates" and (p. 7 l. 22): "In total 12 clone libraries were prepared (2 sites, 2 nodule sizes, and 3 plots)". Therefore there were no analyses of single, individual nodules – in one clone library DNA of microsymbionts from three nodules were present. So, I think that it should be written for example: "...the maximum number of OTUs per group of nodules..." (or per one experimental group or something like this)...

Discussion section, p.14, l. 6-10: "Therefore, higher nitrogen contents of the plants from the 5a site might be attributed to more efficient strains which comprise higher nitrogen fixing activities compared to strains that are associated with nodules of plants derived from the 2a site. To address that question, nifH clone libraries and the influence of soil age on the nodule community were compared." The deliberation about different nitrogen fixing abilities of strains is well-founded, but studying of nifH sequences will not provide an answer for this question – the best method will be plant test. Of course the Authors did not isolate strains but only their DNA, so they are not able to perform plant tests – so the only way to correct it will be not do write about relationships between symbiotic efficiency and diversity of nifH sequences.

Discussion section, p. 14, l. 16-19: "Hence it is very likely that the medium sized nodules represent the most active nodules, which is further underlined by the tendency of nifH copy numbers in nodules of the medium size class mainly from 2a site...". I do not think so – there were more nifH copies in these nodules (fig. 2), but plants from 2a

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site had more medium nodules than plants from 5a site, and in spite of this - nitrogen content of plants from 2a site was lower comparing to plants from 5a site (Tab. 1) – therefore those nodules couldn't be "the most active"

Discussion section, p.15 l. 8-12: "However, one might ask why these nodules did not develop to a medium nodule. Due to low nutrient contents in the soil and the fact that the plants does not spend more energy than needed in nodule production, it is obvious that there might be not enough energy available to promote the maturation of more medium or large nodules." In my opinion this is not supported by presented data – plants grown on "better" soil – 5a (Table 1) had more small and less medium or large nodules (Fig. 1). I think the answer is simpler: not all nodules emerge at the same time; clover produces indeterminate nodules, therefore young, small spherical nodules evolve into mature, larger, rod-like nodules – if there is enough time... Of course spherical nodules might be old and ineffective (and therefore small, not supported by plant with nutrients), but (more likely) they may be effective but young (and therefore small), and only nitrogenase (reduction of acetylene) assay could answer this question.

Table 1 – The abbreviations used in the table (i.e. "DON", "TN" etc.) should be explained in table legend

Interactive comment on Biogeosciences Discuss., 9, 13135, 2012.

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