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Review of the PhD thesis

'The linkage between denitrification activity, N gas em issions, and the size of the denitrifier community in pasture soils'

by Jiri Cuhel

The thesis is comprised of five papers published in international journals of very high reputation. For two of the papers Jiri Cuhel is listed as first author. For three other papers Jiri Cuhel acted as a co-author. The author's contribution to each of these papers (which were written in co-operation with the most prominent groups in the fields) is clearly described on page iii of the thesis and appears to be appropriate.

The *General Introduction and Aims* section is written clearly and the author comes to the point. Not only does it give good background information on the importance of nitrous oxide emissions from pasture soils, on ecophysiology of denitrification and denitrifiers, including their spatial distribution. However, this section remains a bit speculative and no real answer is given concerning the spatial distribution of bacterial communities and nitrous oxide emissions. A link is made to the recent hypothesis of Fierer et al. who have suggested that rather than taxonomic patches, patches of communities classified as K- and r-strategist communities might be the background to particular spatial distributions.

The sampling area within the Borova Farm area is well described in the *Methods* section, and most importantly there are also deep links to other projects carried out in this area. The *Results and General Discussion Section* relates to the five publications. In paper I (Chonakova et al. (2009) different genes related to denitrification were investigated and related to the intensity of land use (cattle impact). In paper II by Phillipot et al. (2009a) in Environmental Microbiology field scale patterns of denitrifiers were determined. The main finding was that the general bacterial community as well as the denitrifier community are controlled by similar external factors. Only the *nirS* gene was correlated with other properties such as nitrate and ammonia concentrations, pH and soil moisture. In contrast to the genes, denitrification enzyme activities were strongly correlated to cattle impact. In paper III (Philippot et al., 2009b) the author investigated more specifically certain bacterial species and genera. The main finding was that different controls act on different bacterial taxa. In this paper, the contribution of Cuhel, however, was restricted to soil sampling and DNA extraction.

In paper IV (Cuhel et al., 2010) investigated interrelationships between pH and denitrifier community abundances, and nitrous oxide emissions. Most importantly, denitrification genes were found not to be correlated with N fluxes in the soils. In a topically slightly distant part of the research, the author found that not distal but proximal pH control apparently is linked to

denitrification activity in pasture soils. Interestingly denitrifying enzyme activity was driven by the long-term pH management rather than short-term pH changes (Cuhel and Simek, 2011).

Altogether, this thesis is a decent collection of own (first-authored) and co-authored papers employing a wide variety of technologies in an emerging and important field of soil microbiology.

Recommendation: Acceptance

Innsbruck, May 9, 2011

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Evaluation of the dissertation of Jiří Čuhel entitled:

The linkage between denitrification activity, N gas emissions, and the size of the denitrifier community in pasture soils.

The thesis consists of summary + 5 research articles. All the articles have been published in international journals of very good quality. Jiří Čuhel is first author on two of these, and third, second, and fifth among 7, 9 and 8 co-authors on the three other papers.

All studies deal with denitrification and the denitrifier community. In the first article a pasture used for overwintering area for cattle is studied. Due to the cattle preferring being near the cow barn, this resulted in a gradient of impact of the cattle. This is a very nice experimental area with a gradient in N content in the soil. The second and third paper used a pasture with different parts being grazed at different intensities (different concentration of cattles) also resulting in a gradient of impact. The two last papers (paper IV and V) are targeting effects of different pH and use an area, where different pH in the soil has been achieved by adding hydroxide or acid.

In paper I the bacterial genes *nirK*, *nirS* and *nosZ* are used as proxies of the denitrifying community and compared with different estimates of N_2O-fluxes. The *in situ* N_2O flux differed depending on time of year, and maximum was also found in different parts of the field. Highest potential denitrification and abundance of genes was found in the most cattle impacted part. Interestingly there was a shift in the N_2O/N_2 ratio depending on cattle impact. This is a very thorough study on large scaled effects, being a starting point for the more fine-scaled studies (paper II and III).

Both in paper II and III the fine-scaled pattern of denitrification is studied. In paper II geostatistics with krieging is used to map the abundance of different genes involved in denitrification, as well as potential denitrification activity. Interestingly there was a good correlation between the abundance of denitrification genes and the total 16S rRNA abundance, suggesting that the abundance of the denitrifier community is not determined to a large extent by the denitrification trait. However, there was a good correlation between the relative abundance of the *nosZ* gene and the $N_2O/N_2O + N_2$ ratio, suggesting that *nosZ* has a potential to be used in modeling denitrification in the soil.

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In paper III the same geostatistical approach as paper II is used, but the study concerns the spatial patterns of some bacterial taxa using qPCR. A strong spatial pattern was detected for most taxa and was correlated to soil chemical properties.

Both paper II and III are mainly descriptive, and although some possible correlations to different environmental factors were found, further experimental evidence for any direct connections are of course needed. However, this type of descriptive studies is important as starting point for further hypothesis testing and as such is of a large value.

Paper IV and V focus on one important environmental factor determining denitrification, namely pH. In paper IV denitrification, and especially the N_2O/N_2 ratio is studied in soils with different pH. Low pH resulted in relatively more N_2O being produced although total denitrification was highest at alkaline pH. As expected the size of the denitrifier community was not correlated to fluxes, except the *narG* gene abundance. I like this paper very much, in that it uses a field experiment changing the pH over long times (10 months). This partly avoids the immediate effects of pH changes on DOC, which usually confounds short term measurements in the laboratory.

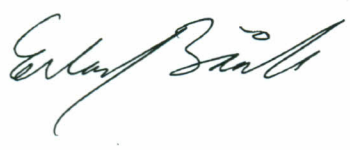
In paper V (a short communication) Jiří Čuhel sets out to differentiate between short- and long-term effects of pH on denitrification rates. In that he uses the long-term pH manipulation experiment from paper IV, but pH is also altered just before measuring potential denitrification activity. The activity was mainly driven by the long-term pH changes, while the short-term change in pH affected the ratio $N_2O/N_2O + N_2$ produced.

Finally, the general introduction I found easy to read, and, besides summarizing the main results, it also presented a good general overview of the subject.

There are two parts in the final evaluation of a PhD thesis: quantity and quality of the work. The quantity of the work presents no problem to me and is well within what is expected of a thesis, even if the large number of authors in some of the paper makes it a bit difficult to evaluate the part which Jiří Čuhel is responsible for. I have even less problems with the quality. All papers to me are of highest international standard (which of course the fact that they are all published in international journal also indicates). Some of the papers can be criticized for being mainly descriptive; however, this type of good data is actually very much needed in soil microbiology. The last paper is a short communication, but the combination of long-term and short-term pH changes actually to me makes it the most interesting paper in this thesis.

Summarizing, I strongly recommend that the thesis of **Jiří Čuhel** entitled "*The linkage between denitrification activity, N gas emissions, and the size of the denitrifier community in pasture soils*" should be awarded a PhD.

Lund 30/5 2011



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Posudek doktorské disertační práce Mgr. Jiřího Čuhela

The linkage between denitrification aktivity, N gas emmissions, and the size of the denitrifiers community in pasture soils

Předkládaná práce Mgr. Jiřího Čuhela, The linkage between denitrification aktivity, N gas emmissions, and the size of the denitrifiers community in pasture soils, se zabývá problematikou denitrifikace a s ní spojenou otázkou emise dusíku a jeho oxidů do atmosféry, i studiem mikrobiálních společenstev podílejících se na tomto procesu. Recyklace N_2 do atmosféry představuje největší terestriální export tohoto prvku v rozsahu zhruba 140 Tg za rok. Jde o proces spojený s bakteriální respirací, kdy je NO_3- využíván mikroorganismy místo O_2 jako terminální akceptor elektronů. Podílí se na něm systém čtyř enzymů, nitrát reduktáza nacházející se ve většině bakteriálních buněk redukující nitráty na nitrity, nitrit reduktázy přeměňující nitrity na volně difuzibilní NO, který je dále redukován nitric oxid reduktázou na N_2O. Poslední krok představuje redukci N_2O na N_2 pomocí enzymu nitrous oxid reduktáza.

Celý proces denitrifikace spolu s opačným procesem nitrifikace i fixace atmosférického dusíku se podílí na koloběhu tohoto významného makrobiogenního prvku v přírodě s řadou významných implikací pro úrodnost půd, možné toxické působení iontů NO_3 a v neposlední řadě pro klimatické změny degradací ozónové vrstvy působením N_2O, jehož koncentrace v troposféře se ročně zvyšuje (nejen zásluhou biologických procesů) o 0,25% a vzhledem ke zhruba 150ti násobné účinnosti absorpce infračerveného záření ve srovnání s CO_2 tak mimo jiné velmi silně přispívá ke skleníkovému efektu. Předkládaná práce tak představuje významný příspěvek ke studiu této vysoce aktuální problematiky.

Disertační práce Mgr. Jiřího Čuhela představuje soubor pěti původních vědeckých článků publikovaných v prestižních mezinárodních časopisech. První práce publikovaná v Soil Biology and Biochemistry v roce 2009 charakterizuje experimentální plochy využívané pro všechny studie a analyzuje složení mikrobiálních společenstev denitrifikátorů v závislosti na zatížení dobytkem. Druhá práce publikovaná v témže roce v Environmental Microbiology provedla podrobné mapování výskytu denitrifikačních společenstev na výzkumných plochách s velice důkladným a zajímavým statistickým vyhodnocením získaných dat. Na ni navázala třetí práce publikovaná v témže roce i časopise, která podrobnou charakteristiku nitrifikačních společenstev doplnila o taxonomické studie založené na genech pro 16S rRNA. V této práci byly také publikované nové velice zajímavé primery specifické pro vybrané skupiny bakterií. Čtvrtá práce publikovaná v následujícím roce v Applied and Environmental Microbiology doplňuje předchozí práce studiem vlivu půdního pH na složení denitrifikačních komunit i produkci N_2O a N_2. Na ni navazuje pátá práce publikovaná v letošním roce v Agriculture, Ecosystems and Environment, která vliv pH na denitrifikační aktivitu dále analyzuje a rozděluje na přímý vliv pH na aktivitu denitrifikačních enzymů a zprostředkovaný vliv přes změny ve složení mikrobiálních denitrifikačních společenstev. Celkově podávají předložené články velice komplexní a ojedinělý pohled na problematiku denitrifikace v podmínkách přirozených travních společenstev a je třeba je hodnotit velice kladně.

K předkládaným pracím bych měl následující dotazy.

Pro extrakci DNA z půdních vzorků byly použity dvě odlišné metody. Zajímalo by mě, co bylo důvodem k jejich použití a shledal-li autor nějaké rozdíly mezi nimi.

K extrakci DNA se váže i mé další otázky:

- Pro posouzení kvantity izolované DNA bylo použito měření na spektrofotometru. Nepovažuji tuto metodu za nejspolehlivější a rád bych znal názor autora na tuto problematiku.
- K čištění DNA byl použit PVPP – rád bych se zeptal na účinnost této metody.
Konečně k manipulaci půdního pH byly použity roztoky KOH a H2SO4 – zajímal by mě důvod pro použitou molaritu roztoků a ocenil bych trochu podrobnější rozbor vlivu na složení vegetačního krytu na pokusných plochách nejen co se týká druhového složení společenstev, ale především trvalosti tohoto efektu.

Celkově považuji předloženo disertační práci za velice zdařilou a přínosnou a doporučuji ji k obhajobě.

Ing. Martin Krsek, CSc.

V Brně 9.6.2011