Nitrification in acid coniferous forests:
Some soils do, some soils don't

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To my parents in heaven

SUMMARY

Nitrification in acid coniferous forests: Some soils do, some soils don't

Nitrification is assumed to be rate-controlled by the activity of ammonia-oxidising bacteria (AOB). Therefore, AOB are considered to play a crucial role in the nitrogen cycle and nitrification in particular, and AOB were the main subject of the microbiological analysis described in this thesis. The general aim of this thesis was to elucidate the relation between the presence of AOB, environmental factors and nitrification rates in the soil layer of acid coniferous forests. Especially, the question why some acid forests showed nitrification while others did not was addressed in this thesis.

This thesis showed that nitrification occurred readily in some acid coniferous forest soils, but slowly in others. *Nitrosospira* cluster 2 was detected as the sole AOB in acid coniferous forest soils that showed high nitrification rates (Chapters 2 to 4). Conversely, AOB communities could not be detected in acid coniferous forest soils with low nitrification rates. The presence of *Nitrosospira* cluster 2 and differences in nitrification rates between nine Scots pine forest soils located throughout the Netherlands and Finland (Chapter 2) and in the Appelscha, the Netherlands forests containing pine, spruce, fir and larch tree species (Chapter 3) correlated with soil C/N ratio. Nitrification and *Nitrosospira* cluster 2 were exclusively detected in acid coniferous forest soils with C/N ratios below 26. Moreover, four tree-specific soils, i.e. pine, spruce, fir and larch forest soils were all sampled near Appelscha and located within a few kilometers from each other (Chapter 3). This indicates that differences in nitrification rates between nine Scots pine forest soils located throughout the Netherlands as well as Finland (Chapter 2) were also not related to geographic distances between these forests.

Atmospheric nitrogen deposition rather than geographical location appeared to be a more important affecting nitrification and the presence of AOB in the nine Scots pine forest soils (Chapter 2). AOB could not be detected and nitrification rates were nearly zero in soils receiving relatively low, according to Dutch standards, atmospheric nitrogen deposition. However, low atmospheric nitrogen deposition was not likely to be the sole factor determining nitrification (Chapter 3). The four different soil types, i.e. pine, spruce, fir and larch forest soils studied there came from around Appelscha, a location with low atmospheric nitrogen deposition. Still, three out of four forest soils revealed nitrification and the presence of AOB.

The low nitrification rates observed for some soils were not caused by the complete absence of AOB nor solely by C/N ratios but also by additional abiotic factors (unfavourable environmental conditions) (Chapter 4). Liming (to increase pH) and liming plus nitrogen amendment increased nitrification in soils that were known to have low nitrification rates. Conversely, nitrogen amendment did not affect nitrification rates in these soils. These results suggested that pH related factors, other than the effect of pH on ammonia availability (described below), had negative effects on the growth of AOB and their associative nitrifying activity in non-nitrifying soils. In soils treated with liming only, an effect of liming on C/N ratio was not observed, but still nitrification increased. On the other hand, when only ammonium was added, C/N ratio dropped but this did not induce nitrification. Presence and changes in the growth of AOB in soils

after treatment with liming and liming plus nitrogen amendment were apparent; not only nitrification rates increased but also AOB could be detected using molecular tools.

However, the low nitrification rates observed for some soils cannot be (solely) explained by unfavourable abiotic soil conditions. Chapter 5 showed that the onset of nitrification was observed after native or sterilised non-nitrifying soils were inoculated with fresh soil or soil slurry from the nitrifying soil. Conversely, when nitrifying soil was inoculated with non-nitrifying soil or soil slurry, nitrification was not inhibited. These indicate that abiotic factors or microorganisms that can potentially inhibit nitrification could not establish themselves in the nitrifying soils. These results falsified the hypothesis that interactions within the non-nitrifying soil microbial community prevent the onset of the growth and activity of AOB. Despite the occurrence of nitrification the bacterial community structure did not change to become nitrifying soil-like, suggesting that minor changes in community structure were sufficient to result in nitrification. General community profiling techniques targeting bacteria appear to be too insensitive to detect these minor microbial populations.

To summarise the effects of biotic and abiotic factors on the occurrence of nitrification in acid coniferous forest soils: presence of AOB, geographical location and ammonia availability are not particular factors that influence the occurrence of nitrification. Microbial interactions, C/N ratio, atmospheric N deposition, tree species and soil pH however do. The effects of microbial interactions, C/N ratio, atmospheric N deposition, tree species and soil pH are also related to other factors.

Very recently, the paradigm of AOB being primarily responsible for nitrification has become under siege. After the discovery of a unique ammonia monooxygenase gene on an archaeal-associated genome fragment in 2004, an ammonia-oxidising Archaea (AOA) has been isolated and it has been shown that AOA occur in high numbers in many habitats, including forest soils. In how far AOA are present and to which degree they contribute to nitrification in more acidic forest soils, such as investigated in this thesis, is unknown. To determine and understand the relative contribution of AOA and AOB to nitrification in acidic forest soils, biochemistry, physiology and ecology of AOA should be examined and compared to that of AOB.