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Drivers for ammonia-oxidation along a land-use gradient in grassland soils

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ABSTRACT

In this study, drivers for ammonia-oxidation and the related microbial communities (ammonia-oxidizing bacteria and archaea) were investigated in grassland soils on the local as well as on the regional scale focusing on the role of land-use intensity (LUI). To this end, 150 sites from three distinct regions across Germany were selected, covering the whole range of LUI levels (from natural grasslands up to intensive managed meadows). Furthermore, the role of contrasting soil types was analyzed in one of the regions (high vs low organic matter content) for ammonia-oxidation. We revealed a significant increase in potential nitrification rates and abundance of ammonia-oxidizing microbes at two sites on the local level from extensively to intensively managed sites, which indicates that the response pattern of ammonia-oxidizing microbes in grassland soils is likely triggered to a large extent by LUI. However at a third site, where two different soil types were investigated, no correlation between LUI and potential nitrification rates was observed, and only a site-specific effect was apparent. At this site, on the one hand the specific soil type (Histosol) and the related continuous nutrient mobilization from the former peat matrix, as well as the high groundwater level, which could induce a high abundance of methane- oxidizing microbes in the top soil, may be of greater importance as a driver for potential nitrification rates and abundance of ammonia- oxidizing microbes than LUI. On the other hand, the mineral soils of this site were characterized by extreme water shortage, which may also explain the lack of potential nitrification and the abundance of ammonia-oxidizing bacteria and archaea. Thus any extrapolation of local data to regional predictions must be made with care, as factors other than LUI may be of importance if the nitrification potential of a soil is to be described.

1. Introduction

Ammonium is the central metabolite of the nitrogen cycle as it links the inorganic to organic phases of nitrogen species. Most organisms (including plants and animals) are able to uptake ammonium and use it for the synthesis of amino acids. Therefore, ammonium availability is strongly linked to the productivity of ecosystems, and can be regarded as an indicator for sustainable use, for example in unfertilized soils (Kowalchuk and Stephen, 2001; Ollivier et al., 2011). Under aerobic conditions in non-fertilized soils the availability of ammonium is mainly controlled by the transformation rates of nitrogen into ammonium via nitrogen fixation and the activity of nitrifiers, which use ammonia as the principal electron donor for autotrophic growth (Stahl and de la Torre, 2012), besides uptake of ammonium for biomass generation and mineralization (the stepwise degradation of dead biomass proteins and polypeptides into ammonium). Thus microbes capable of oxidizing ammonia, which is the first step of nitrification, determine the productivity of ecosystems to a large extent.

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The oxidation of ammonia to nitrite can be performed both by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Treusch et al., 2005), which have been detected in a large variety of habitats ranging from aquatic freshwater ecosystems (reviewed in Kowalchuk and Stephen (2001)) to terrestrial habitats (Fierer et al., 2009; Ochsenreiter et al., 2003; Treusch et al., 2005). Several studies have focused on the respective contributions of AOA and AOB to the ammonia-oxidation in these habitats (e.g. Adair and Schwartz, 2008; Beman et al., 2012; Di et al., 2009; Jia and Conrad, 2009; Leininger et al., 2006; Martens-Habbena et al., 2009; Santoro et al., 2008; Zhang et al., 2010) with partially contradicting observations, which indicate the presence of different drivers of ammonia-oxidizer performance in the investigated environments. Various factors influencing the soil microbial performance of ammonia-oxidizers have been identified so far, amongst the most intense studied are climatic conditions (Tourna et al., 2008), soil chemical and physical properties (He et al., 2007; Nicol et al., 2008; Zhalnina et al., 2012), plant diversity (Kowalchuk et al., 2002) and land-use management. The term land-use intensity (LUI) comprises different management regimes such as fertilization, mowing or grazing which have been demonstrated to affect microbial communities in soils (Nicol et al., 2004; Patra et al., 2006), mainly due to nitrogen-input via fertilizer and manure application or soil compaction by grazing livestock or usage of heavy machinery.

It can be assumed that the land-use intensity (LUI) strongly also drives the abundance, diversity and activity of AOB and AOA in soils. However, as LUI integrates a large variety of factors that influence ammonia-oxidizers to a different degree, such as different fertilization rates, soil compaction or plant species composition, it is not clear if a general link between LUI and ammonia-oxidation can be found, mainly taking into account that site specific conditions like climate or soil type also drive AOA and AOB performance. Overall studies of AOA and AOB dynamics in the response to different LUI levels are rare and mainly cover small scales (Di et al., 2009; Keil et al., 2011; Nicol et al., 2004; Schauss et al., 2009).

In this study we present results from a systematic study on the influence of LUI on AOA and AOB in grassland soils on the local as well as on the regional scale. In this respect, our main focus was to determine overall underlying principles across a large number of different study sites. The selected regions representing the local scale had an area of 300 km^2 each and were located in three areas of Germany (southwest; central; northeast). In each of these regions 50 plots were selected and classified in different LUI levels according to an index of LUI developed by Blüthgen et al. (2012). This index includes the main determining factors of land-use intensity dominating at the study sites, in particular fertilization intensity, mowing frequency and grazing level. At each plot, the abundance of ammonia-oxidizing microbes was measured based on the quantification of the gene coding for the subunit A of the ammonia monooxygenase gene (*amoA*). In addition, potential nitrification rates as well as in situ ammonium levels were determined. To quantify the ammonium input via nitrogen fixation also the abundance of nitrogen fixing microbes was measured based on the nitrogenase gene *nifH*.

We hypothesized an overall positive correlation of LUI and abundance of ammonia-oxidizing microbes and according potential nitrification rates on the local as well as on the regional scale, independent from the soil type and climatic conditions, due to increased ammonium availabilities by fertilizer input or nitrogen fixation in combination with increased soil carbon contents as well as increased oxygen availability in mineral soils. However, very high LUIs could lead to a decrease due to soil compaction by heavy machinery and grazing livestock and less oxygen availability as well as higher competition with the heterotrophic microflora in response to higher nutrient contents. In soils with natural high carbon content, like Histosols, the ammonia-oxidation rates are assumed to be lower independent from the land-use due to the natural high water levels and they do not follow the described trend of mineral soils due to low redox potentials.

2. Materials and methods

2.1. Site description

The experiment was performed in the frame of the 'German Biodiversity Exploratories' (www.biodiversity-exploratories.de; Fischer et al., 2010). The Biodiversity Exploratories are based in three different areas located across Germany: the Biosphere Reserve Schwäbische Alb (ALB) in the South-west of Germany; the National Park Hainich-Dün (HAI) in Central Germany, and the Biosphere Reserve Schorfheide-Chorin (SCH), in the North-east of Germany. Mean annual temperatures have been determined as follows: 6.5–8.0°C (ALB), 8.0–8.4°C (HAI), 6.5–8.0°C (SCH). Mean annual precipitation levels, detected for each region, are the following: 938–963 mm (ALB), 520–600 mm (HAI), 500–800 mm (SCH). Each region comprises 50 grassland study sites, so called experimental plots (EP), so in total our study focused on 150 different grassland plots. For reasons of clarity, the grassland plots were termed as follows: AEG1-50 (ALB region), HEG1-50 (HAI region) and SEG1-50 (SCH region). The Schorfheide-Chorin exploratory comprises mineral soil sites as well as degraded peat soil sites. For reasons of better discrimination, sites of mineral soil were termed as SCH-MB (SCH-mineral soil), peat soil sites were termed as SCH-NM (SCH-peatland). The dominating soil types are Histosols, Cambisols, Glysols, Luvisols and Albeluvisols. The experimental sites of the Schwäbische Alb comprised Cambisols and Leptosols, whereas in Hainich-Dün mainly Cambisols and Stagnosols were found. The respective soil textures are characterized by predominantly sandy textures in the Schorfheide-Chorin exploratory. In Hainich-Dün and Schwäbische Alb exploratory, loamy and clayey textures are found. For further details see Fischer et al. (2010).

2.2. Land-use intensity

On the basis of conducted land-use surveys, Blüthgen et al. (2012) developed an integrative measure for the land-use intensity based on the following components: fertilization, including organic and inorganic fertilizers (in kg nitrogen per ha per year), mowing frequency per year and grazing intensity by cattle, horses or sheep, measured via livestock density (in livestock units per ha per year). The measured intensities of the three components were normalized to the typical forms of management for the respective regions. The calculated LUI index is the sum of all three normalized components and reflects a numerical gradient, reflecting extensive respectively intensive land-use management. Underlying equations for the calculation of the index as well as further information can be found in Blüthgen et al. (2012). The originally proposed LUI index has been based on a three year index, whereas for our studies the recently calculated five-year-index was used based on the same equations (Blüthgen et al., unpublished). To monitor changes across different regions along the land-use intensity gradient, the results were either plotted against corresponding LUI – indices or grouped according to their individual LUI values to meet the demands of a rather detailed gradation on the one hand and the availability of a sufficient number of independent replicates, required for subsequent comprehensive statistical analyses at the same time on the other hand. The levels of LUI were termed with consecutive numbering from 1 (extensive) to 5 (intensive) for reasons of clarity (see also Table 1): Level 1 = LUI indices below 1.0; Level 2 = LUI