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Original article

Livestock grazing on alpine soils causes changes in abiotic and biotic soil properties and thus in abundance and activity of microorganisms engaged in the methane cycle

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ABSTRACT

Three subalpine soils with similar properties and climate conditions, but with different land use were chosen for assessing the abundance and activity of aerobic and anaerobic microorganisms, particularly of methanotrophic *Bacteria* and methanogenic *Archaea*. Within these three soils, there was a distinct gradient in grazing and manure input that ranged from abandoned site (no grazing) to pasture (intense grazing) and included also a meadow (moderate grazing). Activities of dehydrogenase and ammonification indicated higher microbial activities in soil from the abandoned site than in the pasture and in the meadow soil. These differences in microbial activity between unmanaged and managed soils additionally increased during the growing season. Our results show that temperature and soil water content significantly influence methane production and that the grazing gradient is an additional important factor. At 50 °C and under water-saturated conditions, methane production reached 4.42 ± 0.09 ml g^{-1} dry weight in the pasture soil samples after 6 weeks. By contrast, low methane production was detected in soil from the abandoned site, whereas the meadow values were intermediate. Additionally, methanotrophic activities were investigated under an aerobic atmosphere with 5% CH_4 and led to the surprising result that methane oxidation only occurred in undisturbed soils, whereas all sieved soil samples even produced further methane.

1. Introduction

Methane is an important greenhouse gas that absorbs infrared radiation 20–30 times more efficiently than carbon dioxide [21]. According to the Intergovernmental Panel on Climate Change, the methane concentration in the atmosphere increased from 715 ppb in preindustrial times to 1774 ppb in 2005 [13]. Anthropogenic methane sources, such as flooded rice fields, domestic ruminants, sewage treatment, and biomass burning, contribute with 63% to the total global methane emission budget which is in the range of 500–600 TG methane $year^{-1}$ [11].

About 70–80% of the atmospheric methane is produced biologically by obligate anaerobic methanogenic *Archaea* [21]. Methanogens convert $H_2 + CO_2$, methyl compounds, or acetate into methane via methanogenesis, an important process during anaerobic decomposition of organic matter [41]. Studies during the last decades showed that methanogens can inhabit not only anaerobic habitats but also aerobic soils such as cultivated fields, (beech) forests, savannahs, and even deserts [2,20,28,37]. Peters and Conrad [28] suggested that these methanogens survive in anaerobic micro niches, whereas other studies indicate that some methanogens are aerotolerant and thus survive oxic periods within the soil (e.g., Ref. [7]). However, the incidence of methanogens in soils seems to depend on many physical (e.g., temperature) and biological factors (e.g., plant physiology) [6], and the majority of these dependencies is so far not clearly understood. For example, CH_4 production is influenced by nitrogen fertilisers because methanogens compete with nitrate-reducing bacteria for hydrogen and certain organic sources like acetate. Besides, poisonous compounds such as nitrite can be produced during nitrate reduction [6]. If ammonium is sufficiently available, however, methanogens can stop nitrogen fixation and use more energy for growing [6,22]. Ammonium application can also enhance plant growth leading to an increased carbon availability for fermenting processes [6]. Cattle husbandry also influences the abundance and activity of soil methanogens due to a high manure impact. Applied cattle manure can stimulate mineralisation, microbial metabolism, and thus oxygen demand [30].

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Approximately in the same magnitude than CH_4-production several biotic and abiotic mechanisms are known to oxidize methane, altogether forming methane sinks of about 581 TG methane $year^{-1}$. Besides abiotic processes like photochemically oxidation in the troposphere and diffusion into the stratosphere [11,21] the biological oxidation of methane by methane oxidizing bacteria (MOB) consume about 30 TG methane $year^{-1}$ before it enters the atmosphere. Thus the activity of MOB together with the close association with methanogens guarantee a quite low net methane emission rate [12]. Many studies consider upland soils as methane sinks of atmospheric CH_4. For example, Abell et al. [1] showed that grazing influences the activity and diversity of methanotrophic bacteria in alpine meadow soils. Furthermore, ammonium can competitively inhibit CH_4 oxidation, but the complex relationship between nitrifiers and methanotrophs is not clearly understood so far, and other ecological factors such as acidity have to be considered too [29]. However, studies concerning the effect of nitrogen fertiliser on both methanogens and methanotrophs led to contradictory results [10,21,43] pointing to the necessity of further investigations.

Land management, such as cattle husbandry, influences physicochemical and microbiological properties in the soil which indirectly influence soil methanogens and methanotrophs. For example, land use can affect soil pH, electrical conductivity, and nutrient leaching [3]. Previous assessment of soil organic carbon dynamics at our study site (an alpine pasture with different life stock – see Section 2.1) showed that carbon accumulation in labile SOM (soil organic matter) pools is influenced by land management, whereas the stable SOM pool is more independent [25]. Cattle slurry applications on grassland over 38 years promoted heterotrophic nitrification, led to an NO_3^- increase and that more labile N sources – instead of recalcitrant organic N sources – were mineralised [27]. The microorganisms engaged in methane cycle are especially affected within habitats that are influenced by climate warming as known for instance for permafrost soils of cold regions [40]. As alpine soils are in various connections similar to soils from arctic sites [24] investigations concerning methanogenic and methanotrophic microorganisms are necessary but still rare.

Therefore the main objectives of this study were to assess: I) the impact of land management on physicochemical parameters in Alpine soils with similar properties and preconditions, II) the effect of land use on soil microbiological properties in general and on the abundance and activity of methanogenic and methanotrophic microorganisms in particular, and III) whether changes in methanogenic and methanotrophic properties can be drawn back to changes in physicochemical and microbiological properties.

2. Materials and methods

2.1. Study sites and sampling procedure

The study sites are located in the Central Alps near the village Neustift, Tyrol (47°07′4.1″N, 11°19′18.7″E) at the Kaserstattalm. Three sites in immediate distance to each other were chosen: I) an abandoned site (A) which has been lain fallow since 1983, II) a meadow (M) which has been cut annually in July, fertilised every 2–4 years, and grazed in late summer by cattle since 1990, and III) a pasture (P) which has been grazed by cattle from June to September since 1998 [25]. Thus there is a distinct increase in grazing intensity and manure input from abandoned (no grazing) to meadow (moderate grazing) to pasture (intense grazing) sites. Information on soil properties and climate conditions is available elsewhere [4,25,34].

Soil samples were collected twice in July and in October 2010 just underneath the root zone at a depth of about 10 cm. At least 15 subsamples were taken from each site within an area of about 100 m^2 and were merged to one mixed sample for each site. Additionally, core samples ($Ø = 5$ cm, $V = 100$ cm^3), which included plant roots, were taken for further investigations. All samples were stored at 4 °C on the same day. On the next morning, the spade collected soil samples were sieved (2 mm) and stored at 4 °C.

2.2. Chemical analyses

The soil dry weight was determined after the samples were dried at 105 °C overnight. The organic matter content in the soil was assessed in dried samples that were incinerated at 430 °C for 5 h. A temperature of 430 °C was used to avoid gaseous losses of CO_2, derived from the high carbonate content of the soil. Soil pH was determined electrochemically in a 0.01 M $CaCl_2$-solution. Plant available ammonium was extracted with a 2 M KCl solution and measured spectrophotometrically in a solution containing NaOH, nitroprusside, and dichloroisocyanuric acid sodium salt [33].

2.3. Soil respiration and microbial biomass (SIR) measurements

For soils respiration and microbial biomass measurements, an automated system with an infra-red CO_2 analyser was used [16]. This system allows hourly CO_2 measurements of up to 24 soil samples in defined intervals under a controlled air flow [16]. The same soil samples were subsequently used for the determination of the microbial biomass, based on the substrate induced respiration (SIR) method [33].

2.4. Enzyme analyses

Activities of dehydrogenase (DH) were determined by incubating the soil samples with triphenyltetrazolium chloride (TTC) for 16 h. The produced TPF was measured colourimetrically via photometry at 546 nm [33]. For the determination of the ammonification (AM) activity, soil samples were saturated with water for 7 days, and the ammonium released was measured according to Schinner et al. [33]. For determination of DMSO reduction, an important step within the S-cycle, soil samples were incubated with DMSO which can be reduced to dimethylsulfide (DMS) by both, anaerobic and aerobic microorganisms during the incubation. DMS, gaseous at room temperature, was subsequently measured via gas chromatography [33].

2.5. DNA extraction and quantitative PCR (qPCR) of soil Archaea

The DNA was extracted in duplicates with the commercially available kit (*NucleoSpin Soil*, *Macherey–Nagel*). The sample purity and DNA quantity of the extracts was subsequently measured spectrophotometrically (see Section 2.9). For qPCR analysis of the *Archaea*, autumn soil samples were used in duplicates, applying the primer pair 787F/1059R [42] with a concentration of 200 nM per reaction. The SensiFAST™ SYBR No-ROX kit (*Bioline*) was used for detection. PCR conditions, based on the work of [42]; were modified as follows: an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 20 s, annealing at 61 °C for 20 s, and extension at 72 °C for 20 s. Melt curve analysis was applied for checking the PCR products after quantification. *Methanosarcina acetivorans* was used as DNA standard. Furthermore, a negative control containing DNA of *Escherichia coli* and a non-template control containing DNase/RNase-free distilled water (*UltraPure*™) were included.