Factors affecting the localization of transformations of nitrogen and methane in soils

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ABSTRACT

Biological, chemical, and physical factors regulate the growth and activity of bacteria responsible for nitrification, denitrification, and methane oxidation in soils. The restriction of activities to certain soil horizons or to certain microenvironments can affect important environmental processes including losses of fertilizer nitrogen, and the production and consumption of the atmospheric trace gases nitric oxide, nitrous oxide and methane.

Introduction

Among the many factors likely to govern the growth and activities of microorganisms involved in transformations of nitrogen and methane, the most important are the availability of substrates (organic or inorganic reductants) and electron acceptors (principally O_2), the presence of potential inhibitory compounds, and the ambient pH of the environment. Here we consider these in relation to N transformations, mainly nitrification and denitrification, and CH_4 oxidation.

Substrate effects

Nitrification is limited by the availability of NH_4^+ in some soils, but CO_2, the source of carbon is rarely limiting. Denitrification was recognized long ago to be frequently limited by available carbon, but it was Parkin [18] who demonstrated the importance of active "hot spots", located in or around fragments of organic matter, in causing the high spatial variability often found in soil assays of denitrification. Later microsensor studies by Højberg et al. [12] determined both N_2O and O_2 in large water-saturated aggregates on the surface of which a disc of clover leaf was pressed. Incubations in the presence and absence of C_2H_2 demonstrated clearly that in the region below the disc, leaf components stimulated both O_2 consumption and denitrification to levels that were much greater than were observed in zones well outside the leaf disc.

Methanotrophic bacteria responsible for oxidation of CH_4 are widely distributed in nature. They are especially active adjacent to methanogenic habitats, such as in the surface layers of peats and in landfill cover soils. In these environments, the bacteria are exposed to relatively high CH_4 concentrations and thus the so-called low affinity (high K_m) methanotrophs seem dominant throughout the aerobic zone. On the other hand, many aerobic soils exhibit CH_4-oxidizing activity and consume atmospheric CH_4, but despite the fact that the source is the atmosphere, this activity is frequently (especially in forest soils) restricted to a subsurface layer. These bacteria are of the high affinity (low K_m) type, consuming CH_4 at concentrations two or three orders of magnitude lower than exist in the low affinity systems [11]. It is not clear how such methanotrophs can show maintenance and

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growth at such low CH_4 concentrations unless some other C_1 substrate, such as methanol, is at least transiently available. Possible reasons for their restriction to the subsurface layer are discussed later.

Inhibitor effects

Polyphenols and tannins have been suggested as inhibitors responsible for the little or no nitrification occurring in some soils [17]. Monoterpenes are released from some forest systems at rates up to 5 mg m^{-2} h^{-1} [13] and have been implicated, by Rice and White and their coworkers, in affecting nitrification in forest soils [21,24]. However, the topic is controversial, and Bremner and McCarty [7] claimed that the apparent inhibition seen in earlier studies was due to immobilization of NH_4^+ and/or NO_3^- by microbial activity stimulated by the organic carbon added in the form of the monoterpene. More recent work by Ward et al. [23] shows conclusively that several monoterpenes (especially α-pinene and R+-limonene) inhibit oxidation of NH_4^+ by *Nitrosomonas europaea* in pure culture under NH_4^+-sufficient conditions (Table 1). Both immobilization and inhibition may occur in some systems.

Table 1. Effect of monoterpenes on the oxidation of NH_4^+ by *Nitrosomonas europaea* (from [23]) and of CH_4 by *Methylosinus trichosporium* and some forest soil samples (from [3,5])

	NH_4^+ oxidation	CH_4 oxidation			
	Nitrosomonas europaea	*Methylosinus trichosporium*	Mixed 7-12 cm	Pine 5-10 cm	Aspen 5-10 cm
Addition	10 μg/mL	245 μg/mL	10 mg/g	10 mg/g	5 mg/g
None	100	100	100	100	100
α-pinene	11	4	0	4	50
R+-limonene	35	16		39	35
α-terpinene		75	0	30	34
ß-myrcene	45	93	16	38	85
α-pinene oxide		103	235		
±-linalool		32	116		
α-terpineol		52	136		

Mixed denotes a mixed hardwood stand

We have speculated that the lack of CH_4 oxidation in the surface layer of many forest soils may be caused by a similar effect, and, indeed, extracts of such layers do inhibit CH_4 oxidation in both soils and pure cultures [2]. We have also shown that CH_4 oxidation in some forest soil samples is inhibited significantly by α-pinene, R+-limonene, α-terpinene, and ß-myrcene [3], and that some of these compounds also inhibit CH_4 oxidation by *Methylosinus trichosporium* in pure culture [5] (Table 1). The concentrations used in these studies were fairly ecologically relevant. However, conclusive proof that this effect is responsible for the observed localization of nitrification or CH_4 oxidation in a particular natural system remains difficult to obtain.

pH effects

The autotrophic nitrifiers are notoriously acid-sensitive, and, in pure culture, both they and the known methanotrophs have pH optima near neutrality. Despite these facts, both groups are reported to be active in acidic forest soils. In such reports, it is usually assumed that nitrification activity that is sensitive to C_2H_2 can be attributed to autotrophic rather than heterotrophic nitrifiers. However, acid tolerant NH_4^+ oxidizers have not been isolated from such soils, and it is often speculated that more alkaline microsites in the soil are involved. More recently, de Boer and coworkers [9] pointed out the possible significance of aggregations of cells of *Nitrosospira* spp. surrounded by cells of *Nitrobacter* in a nitrifying Douglas fir soil with a pH of 3.7. Thus it appears that this may be an example of a common phenomenon in which colonial growth of microorganisms protects them from unfavourable environmental conditions.

Methanotrophic activity that occurs in acidic peats and forest soils may be subject to microsite protection of some kind, it may be due to colonial aggregations not so far reported, or it may involve different methanotrophs having different pH optima. We showed that methanotrophs active in peats were not well adapted to the ambient pH and slurries showed optimum pH values that were 0.5 to 1.0 unit higher than the *in situ* pH [10]. We studied this aspect also in forest soils by measuring atmospheric CH_4 uptake rates at a range of pH values both in soil slurries and in bacteria extracted from the soil by Nycodenz density gradient centrifugation [19] and then washed. We found that the pH optima of slurries were generally rather close to the ambient soil pH, but that extracted bacteria showed pH optima for CH_4 uptake that were higher, in the range 5.8 to 6.0, but still about one pH unit lower than the pH optima for two known methanotrophs studied, *Methylobacter luteus* and *Methylosinus trichosporium* [6]. Indeed, for the extracted bacteria, the decline in activity on each side of the optimum was very steep, suggesting that they are very sensitive to even small changes in pH, and that they would be unlikely to grow well in the culture media normally used for the known methanotrophs.

It also appeared in our study that the indigenous methanotrophs in the acidic soils were roughly 50% resistant to the high speed blending used before extraction, but that methanotrophs in neutral agricultural soils were completely inactivated by blending, and no activity could be extracted. However, in the acidic previously-inactive surface horizon of a mixed hardwood soil enriched by exposure to 5% CH_4 the newly active methanotrophs were completely resistant to blending, and, furthermore, about 50% of the activity could be extracted [6]. These observations, all taken together, suggest that the atmospheric CH_4 consumers active in acidic forest soils are different from, or in a physiological state different from, those that are enriched by exposure to high CH_4 concentrations and from the known methanotrophs of laboratory culture.

O_2 effects

Denitrifying reductase genes are repressed by O_2 and their activity is generally inhibited by O_2, so denitrification is mostly limited to anaerobic environments. Recent reports of aerobic denitrification in cultures [15] have left open the question of the ecological significance of this phenomenon. However, the microsensor studies of Revsbech and coworkers have made it possible to determine microprofiles of O_2 and denitrification substrates and products in a range of soils, sediments, and biofilms. They reported that at no time was there N_2O evidence that denitrification occurred in the aerobic zone at > 15 μM O_2 [8].

The microscale distribution of methanotrophs and their activity in soil is difficult to study. Stratified lakes, however, often show a thin plate of activity 0.25 to 1.0 m deep in the metalimnion and close to the aerobic-anaerobic interface. It is suggested that nitrogen limitation could require microaerophilic N_2-fixing growth, but our laboratory studies with N-sufficient cultures of three methanotrophs showed no evidence of microaerophilism [20]. Furthermore, our studies with diffusion-controlled growth in semi-solid agarose columns (Fig. 1) reproduce the type of stratification observed in lakes. Inoculation of an entire column with soil suspension results in the establishment of a band of methanotroph biomass and activity where the tails of the linear gradients of O_2 and CH_4 meet, whether the mineral salts medium contains sufficient NO_3^- (NMS) or sufficient NH_4^+ (AMS) as N source. Based on the concentration gradients, the supply rates (and therefore consumption rates) are generally in the ratio of 1.5-1.9:1 O_2:CH_4 in agreement with other pure culture studies [1]. Simulation of growth in such columns, using the programme of Koch [14], but inserting methanotroph parameters from the literature, reproduces the position of the growth band and the concentration gradients of O_2 and CH_4 seen in the experimental column.

It is noteworthy that nitrogen transformations are often associated with the growth of methanotrophs. For example, in NMS columns, N_2O is produced by associated denitrifiers, and in AMS columns, NO_2^- and NO_3^- may be produced, either by methanotrophic nitrification or by associated autotrophic nitrifiers during the period of O_2 depletion in the column [1,4].

A perplexing phenomenon in relation to O_2 is the frequent occurrence of obligate aerobes in anaerobic environments. For example, potential activities and counts of both nitrifiers and methanotrophs occur in a lake sediment for several centimetres below the one mm or so O_2 penetration depth [22]. They could be transported by bioturbation and are known to survive periods of anaerobiosis, but maintenance and growth for long periods under anaerobic conditions is so far not yet explained. This is but one example of questions that are still open in relation to these interesting organisms.

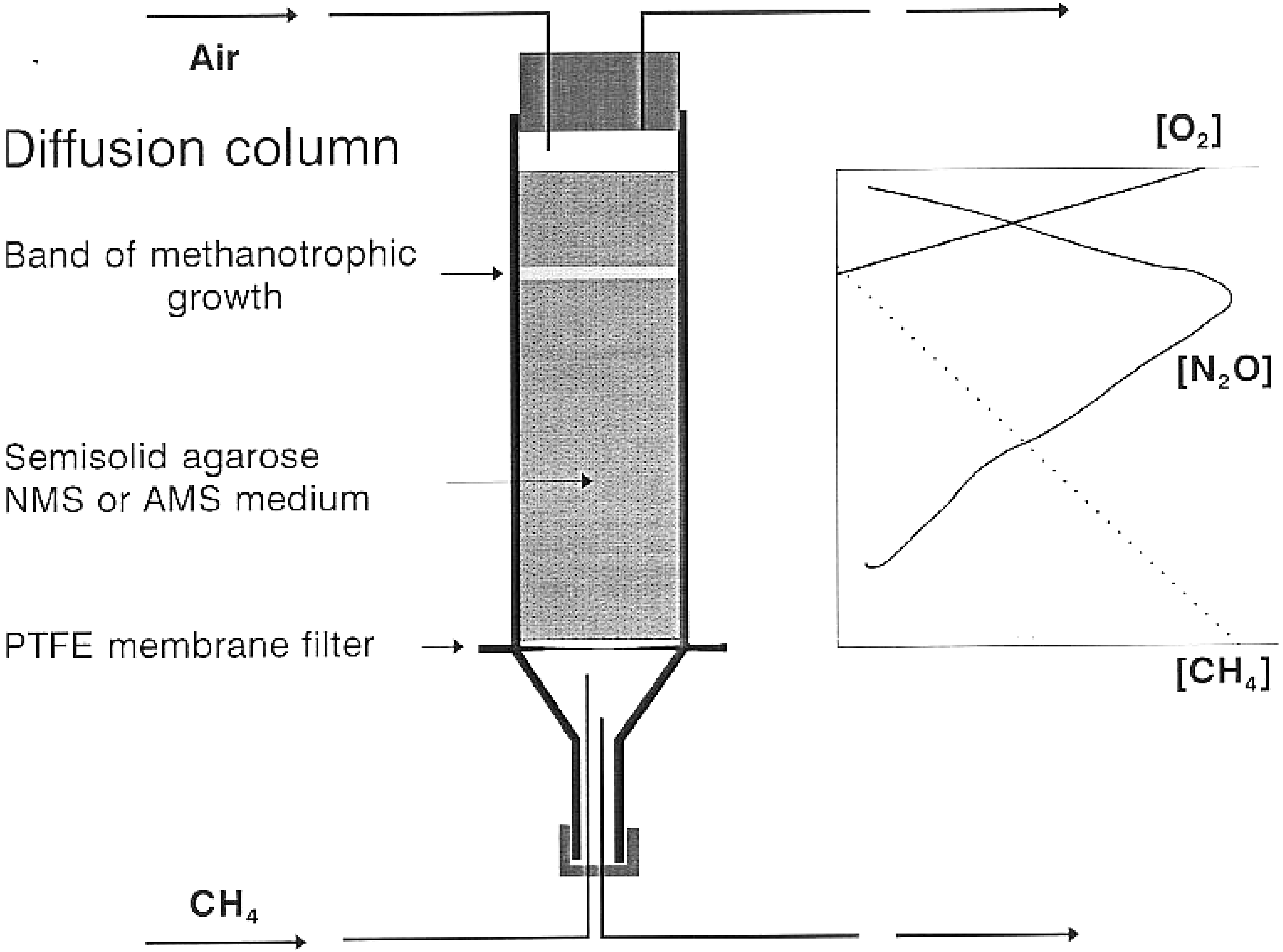

Fig. 1. A gel-stabilized column for studying the growth of methanotrophs in counter-gradients of O_2 and CH_4 (For details, see refs. [1] and [4]).

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