

## Microbial community structure and global trace gases

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### Abstract

Global change can affect soil processes by either altering the functioning of existing organisms or by restructuring the community, modifying the fundamental physiologies that drive biogeochemical processes. Thus, not only might process rates change, but the controls over them might also change. Moreover, previously insignificant processes could become important. These possibilities raise the question 'Will changes in climate and land use restructure microbial communities in a way that will alter trace gas fluxes from an ecosystem?' Process studies indicate that microbial community structure can influence trace gas dynamics at a large scale. For example, soil respiration and CH<sub>4</sub> production both show ranges of temperature response among ecosystems, indicating differences in the microbial communities responsible. There are three patterns of NH<sub>4</sub><sup>+</sup> inhibition of CH<sub>4</sub> oxidation at the ecosystem scale: no inhibition, immediate inhibition, and delayed inhibition; these are associated with different CH<sub>4</sub> oxidizer communities. Thus, it is possible that changes in climate, land-use, and disturbance regimes could alter microbial communities in ways that would substantially alter trace gas fluxes; we discuss the data supporting this conclusion. We also discuss approaches to developing research linking microbial community structure and activity to the structure and functioning of the whole ecosystem. Modern techniques allow us to identify active organisms even if they have not been cultivated; in combination with traditional experimental approaches we should be able to identify the linkages between these active populations and the processes they carry out at the ecosystem level. Finally, we describe scenarios of how global change could alter trace gas fluxes by altering microbial communities and how understanding the microbial community dynamics could improve our ability to predict future trace gas fluxes.

*Keywords:* biogeochemical cycles, decomposition, global change, methane, microbial communities, trace gases

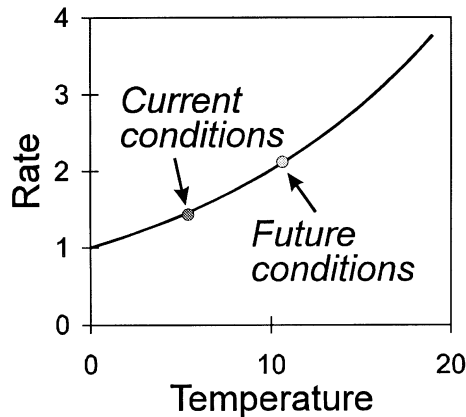
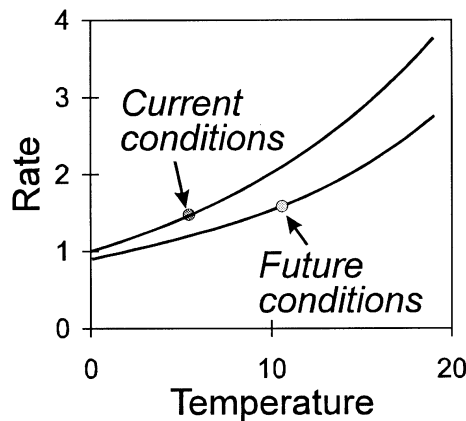
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Critical processes controlling biogenic trace gas (e.g. CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O, and NO) fluxes between soils and the atmosphere are carried out by microorganisms. Understanding trace gas dynamics therefore requires understanding the physiology and population dynamics of the microbes involved. It is not our intention, however, to review microbial trace gas metabolism, as several reviews are available (Bédard & Knowles 1989; Conrad 1995, 1996; King 1997). Rather, our goal is to establish a conceptual and experimental framework for understand-

ing how microbial community structure affects trace gas fluxes and how global change may alter them. This topic has received little attention, in part because of a common assumption that microbial community structure has little relevance to large-scale biogeochemical models (Schimel 1995). Here, we argue that there are cases where microbial community structure is relevant to understanding trace gas dynamics at the ecosystem and larger scales. Additionally, we discuss approaches to effectively integrate studies on microbial population dynamics with biogeochemical modelling.

Global change can affect soil processes in two ways. First, it can alter the functioning of existing organisms. For

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**Community unchanged:****Community changed:**

**Fig. 1** Example of how the relationship between a process rate and an environmental parameter might change depending on whether the microbial community changes or not.

example most microorganisms grow and use substrates faster at higher temperatures; thus, the rates of processes might change, but not their fundamental behaviour or control (Fig. 1a). Second, global change can restructure the community of organisms present. By doing so, it may modify the key physiologies that drive biogeochemical processes. Thus, not only might process rates change, but their fundamental controls could change (Fig. 1b), or a process could be eliminated altogether. Moreover, restructuring the microbial community could introduce or accelerate processes that were previously insignificant. For example, new pathogens might invade an ecosystem (Lonsdale 1994), the lignin-degrading community might become dominated by N-insensitive fungi (Kaal *et al.* 1993), or the decomposer community might shift to organisms with different temperature sensitivities (Fig. 1). For global modelers, these are important issues, as it may be very difficult to predict how changes in biotic communities will alter the functional relationships

between environmental conditions and biogeochemical processes.

Given the likelihood of community changes, to adequately predict how the rates of an ecological process will change with climate, land use, and resource availability we need to know:

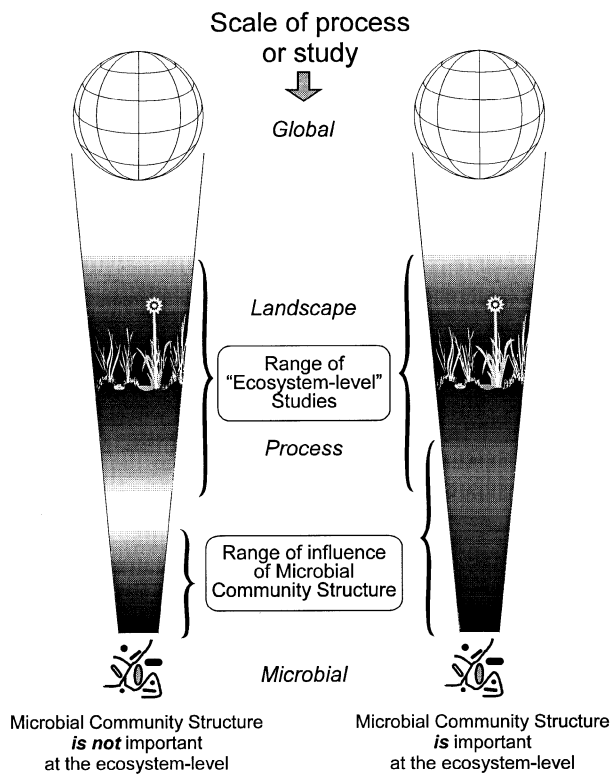
- 1 How many different populations participate in the process and how they vary physiologically (e.g. different kinetics or controls, ability to adjust to altered conditions, etc.);
- 2 How the relative abundance and activities of these populations will change under altered environmental conditions.

Finally, we need to use this information to build quantitative, ecosystem-scale models that link microbial community dynamics with the biogeochemical functioning of the community, and thereby predict changes in microbial processes associated with global change. While plant ecologists have been making advances with this difficult task (Hobbie 1992), microbial ecologists have just begun to address the linkages between community structure and function.

It has actually been hard to show that microbial community structure is *ever* important to consider in ecosystem-scale dynamics. At a very small scale, microbial community structure is *the* critical control on the processes occurring. As the scale increases, however, process dynamics integrate across communities, and their individual influences diminish (Fig. 2). At a very large scale therefore microbial community structure would be unlikely to exert a measurable influence on process dynamics. The scale at which the influence of microbial community structure becomes insignificant varies among ecosystems and processes. For addressing the effects of climate change, CO<sub>2</sub> increase, or land-use change on trace gas biogeochemistry, the appropriate scale of study is the ecosystem. Thus, we can frame two fundamental questions to be addressed in this paper:

- Does microbial community structure have a measurable influence on trace gas fluxes *at the ecosystem scale*?
- Will changes in climate and land use restructure microbial communities in a way that will alter ecosystem trace gas fluxes?

For microorganisms, there are two ways that communities might change that are identifiable and relevant to ecosystem biogeochemistry: (i) whole functional groups (e.g. denitrifiers or methanogens) could be eliminated or introduced, or (ii) there could be functional changes within a group, thereby potentially altering process controls at the ecosystem scale. A third type of community change would be shifts among physiologically 'equivalent' organisms within a functional group. In this case community structure would change, but community functioning would not, and the biogeochemical effects would



**Fig. 2** Framework for deciding which kinds of ecological processes require understanding microbial community structure in order to understand ecosystem-level dynamics. (From Schimel 1995.)

not be measurable. To predict the effect of global changes in climate and land use on the major trace gases, we suggest a six step approach:

- 1 Determine whether there are critical functional groups of microorganisms that are likely to be effectively introduced or eliminated;
- 2 Determine whether physiological differences exist within a functional group that could cause measurable changes in the rates or control dynamics of processes at the ecosystem scale;
- 3 Identify the specific populations within a group that are associated with the different physiologies;
- 4 Determine how the different populations are distributed in nature based on climate, ecosystem structure, and soil properties;
- 5 Determine how community structure will change in response to environmental changes;
- 6 Build process models that incorporate how global change factors drive community differences and changes.

Each of these steps represents a major phase of interdisciplinary research requiring close collaboration between microbial ecologists and ecosystem scientists. Although little research has explored the linkages between microbial communities and ecosystem processes, a few recent stud-

ies provide sufficient information to address steps 1 and 2 for some biogeochemical processes. We believe that the tools needed to begin seriously addressing steps 3 and 4 are now available, but that addressing steps 5 and 6 in an ecologically sound manner remains an achievable (Pace 1996) but relatively distant goal.

We have three objectives for this paper. First, we will attempt to assess the current state of progress for the major greenhouse gases ( $\text{CO}_2$ ,  $\text{CH}_4$ , and  $\text{N}_2\text{O}/\text{NO}$ ) in the context of our six-step approach. Second, we will outline an experimental strategy for completing the remaining steps. Third, we will present several hypothetical scenarios exploring how global change may affect microbial communities and the ecosystem processes they carry out.

### Carbon dioxide

Though we often discuss  $\text{CO}_2$  production as a single process, in reality it results from many distinct microbial metabolic processes, each with its own controls, and no single process necessarily limits the rate of  $\text{CO}_2$  production. In most ecosystems therefore  $\text{CO}_2$  production is a physiologically 'broad' process, and changes in microbial community structure likely exert little influence on ecosystem  $\text{CO}_2$  production (Schimel 1995). From available data, arguments can be made either to support or refute this prediction.

#### *Step 1. Elimination or introduction of functional groups.*

It is a truism of ecology that an ecosystem contains a complete suite of organisms capable of decomposing organic matter to close the C cycle, so a change in the average climate of a biome would not eliminate whole groups of organisms responsible for driving soil  $\text{CO}_2$  fluxes. It may be possible, however, that the diversity and sizes of populations within functional groups may be reduced, at least temporarily, by climatic stresses such as drying/rewetting or freeze/thaw (Clein & Schimel 1994; Schimel & Clein 1996; Young *et al.* 1998, this volume). In systems experiencing regular stress events, the actual rate of a process may be controlled less by substrate availability (as most models assume) and more by the size of the active population, which would be controlled by the balance of stress-induced death and population regrowth. During ecosystem change, microbial population fluctuations may determine at least the transitional rates of soil  $\text{CO}_2$  production. The role of episodic stress and the changes in patterns of stress events is an area of global change research that deserves greater emphasis. A related issue is the effect of pollution on microbial communities. These have the potential to change microbial populations in substantial and unpre-

dictable ways relative to trace gas fluxes (Goldman *et al.* 1995).

Changes in land use may substantially reduce the abundance and activity of different functional groups of soil organisms (Young *et al.* 1988, this volume; Wardle *et al.* this volume). Soil faunal populations may be particularly sensitive to land-use changes. Eliminating grazing on a range of grasslands altered soil trophic structures, particularly reducing fungal-feeding collembola, and significantly changed soil CO<sub>2</sub> fluxes (Bardgett *et al.* 1996). Changes in earthworm and termite populations due to land-use change also occur (Coleman & Crossley 1996) and can cause changes in organic matter decomposition (and hence CO<sub>2</sub> and possible CH<sub>4</sub> emissions; Young *et al.* 1998, this volume).

### Step 2. Changes within groups

Most available data suggest that CO<sub>2</sub> fluxes are insensitive to differences in microbial community structure. Perhaps the most critical evidence for this comes from ecosystem models (e.g. Parton *et al.* 1987; Rastetter *et al.* 1992; Grant *et al.* 1993). These use different approaches, but effectively model organic matter turnover at scales ranging from days and grams of soil (e.g. Grant *et al.* 1993) to centuries and entire biomes (Parton *et al.* 1987). That such models reasonably describe soil C dynamics across a range of soils, vegetation types, and seasons without reparameterizing the basic responses suggests that soil respiration is relatively insensitive to natural variations in microbial communities. Another line of evidence suggesting limited influence of microbial community structure includes studies showing that various simple organic compounds are processed similarly among different soils (e.g. Sugai & Schimel 1993).

Thus, there is evidence suggesting that soil respiration should be insensitive to community structure, but there is evidence to the contrary. For example, Neff *et al.* (1996; Fig. 3), found that respiration in soils from different areas had different temperature responses. Since conditions were held constant in the experiments, and the incubations were too short to allow consumption of the labile C, it is hard to explain these results except by assuming microbial communities with different temperature responses. One could argue that the soils had different organic matter compositions and therefore required different enzymes for decomposition (which could have different temperature sensitivities; Linkins *et al.* 1984). It is likely, however, that different enzymes would be produced by different organisms.

While it is unclear whether soil respiration is sensitive to altered microbial community structure, CO<sub>2</sub> release from decomposing litter is likely to respond to changes in the decomposer community (Schimel 1995). Litter is

### Distribution of Q10 values among tropical soils

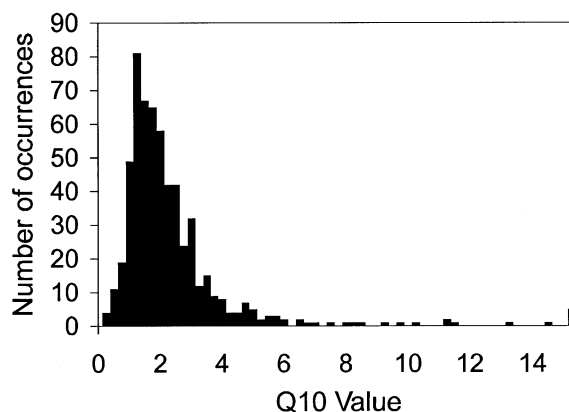


Fig. 3 Distribution of Q10 values among a range of tropical and subtropical soil. Data are from Neff *et al.* (1996).

more chemically defined than soil organic matter, and the rate of cellulose and lignin breakdown may be rate limiting steps for litter breakdown (Sinsabaugh & Linkins 1993). There is a relatively limited community of microbes responsible for these steps. Thus, any change in the composition of the cellulolytic and lignolytic community is likely to have a large affect on overall community activity.

So, how microbial composition influences ecosystem-level CO<sub>2</sub> flux remains unresolved. It seems possible that changes in microbial communities could alter soil organic matter processing, but our current knowledge of microbial community dynamics in soil is too limited to make general predictions. Much more data effectively linking microbial community structure and activity in regard to soil C dynamics are needed. In the context of global change, the effects of CO<sub>2</sub> on microbial populations may be as important to consider as effects of climate patterns. Altered quantity and quality of C flow below-ground resulting from elevated CO<sub>2</sub> may affect both microbial biomass and community structure (Sadowsky & Schortmeyer 1997)

### Step 3. Linking population and process

For CO<sub>2</sub>, work at this level is barely beginning. A useful approach to identifying relationships between processes and communities is suggested by the work of Sinsabaugh & Linkins (1993) and others (e.g. Perez & Jeffries 1990; Kshattriya *et al.* 1992). These workers have developed models of litter breakdown based on specific enzyme activities. It might be possible to link the enzymes with the organisms producing them, and thus link microbial community structure to litter decomposition. Whether this approach can be expanded beyond litter is unclear, as there may not be identifiable rate limiting steps in the breakdown of soil organic matter. Soil organic matter

breakdown is thought to be more heavily dominated by long-lived extracellular enzymes and by free radical processes (Stevenson 1994), and may therefore be somewhat beyond the control of the current microbial community.

### Methane

Unlike CO<sub>2</sub>, CH<sub>4</sub> cycling is dominated by 'narrow' physiologies (Schimel 1995). Methane is produced by methanogens, a group of anaerobic *Archaea* (Whitman *et al.* 1992) that use a limited range of substrates (primarily H<sub>2</sub>/CO<sub>2</sub> and acetate) and a common set of enzymes. They are anaerobes and require very low redox potentials (Paul & Clark 1996). Thus, CH<sub>4</sub> is produced in substantial quantities only in flooded soils, including natural wetlands and rice paddies (Houghton *et al.* 1995). Methane is thought to be oxidized in soil primarily by the bacteria known as methanotrophs (Lidstrom 1992; Steudler *et al.* 1996). Known methanotrophs fall into two distinct groups of aerobic gram-negative bacteria that use CH<sub>4</sub> as their sole C and energy source (Lidstrom 1992; Brusseau *et al.* 1994). They all use a single enzyme to oxidize CH<sub>4</sub> (particulate methane monooxygenase), though a few can also produce a soluble methane monooxygenase (DiSpirito *et al.* 1992). Methane oxidation occurs in most aerated soils (Reeburgh *et al.* 1993). In flooded soils, methanotrophs live in aerated surface and rhizosphere soils and use CH<sub>4</sub> diffusing from the anaerobic zone; these methanotrophs may be exposed to CH<sub>4</sub> concentrations of up to 40% or greater of the dissolved gases (Byrnes *et al.* 1995). In unsaturated, freely drained soil (referred to here as 'upland' soil), the atmosphere may be the only CH<sub>4</sub> source, in which case CH<sub>4</sub> oxidizers are exposed to a maximum of 1.7 ppm CH<sub>4</sub> (equivalent to a solution concentration of about 2.5 nM). Although upland methanotrophs have a much higher affinity for CH<sub>4</sub> than wetland methanotrophs, they are strongly substrate limited (Conrad 1996). It is likely that wetland and upland methanotrophs differ in a number of ways, although we still know little about the organisms that consume atmospheric CH<sub>4</sub> (Conrad 1996).

There is evidence that NH<sub>4</sub><sup>+</sup> oxidizers may also consume CH<sub>4</sub> (Steudler *et al.* 1996; Willison *et al.* 1996). Because NH<sub>3</sub> and CH<sub>4</sub> are similar (Schimel *et al.* 1993), ammonia monooxygenase (AMO) in nitrifiers can oxidize CH<sub>4</sub>, and is physiologically and evolutionarily related to particulate methane monooxygenase (Holmes *et al.* 1995a). Thus, though nitrifiers widen the range of organisms that can oxidize CH<sub>4</sub>, the process remains physiologically narrow.

#### Step 1. Introduction or Elimination of Groups

For CH<sub>4</sub> production, the prime control over whether methanogens are present and active is soil redox potential.

Methanogenesis occurs in essentially all ecosystems that are flooded with freshwater. Only soil drainage and sulphate inundation have the potential to effectively eliminate methanogenesis. Drainage oxygenates the soil and so can reduce or inactivate methanogen populations. Sulphate inputs, such as occur from acid rain or sea water intrusion, inhibit methane production (Nedwell & Watson 1995). Methanogen populations can be increased greatly in an ecosystem by flooding, such as occurs when rice paddies are established.

Methane consumption is ubiquitous; thus, it seems unlikely that climate change would eliminate CH<sub>4</sub> oxidizers. A number of land-use changes, however, have been shown to substantially reduce the activity of atmospheric CH<sub>4</sub> consumers, presumably by altering population size (King 1992; Gullede *et al.* 1997). Nitrogen additions (such as chronic fertilization) have been shown to greatly reduce CH<sub>4</sub> consumption in a wide range of ecosystems (Hütsch *et al.* 1993; Steudler *et al.* 1996; Gullede *et al.* 1997). Salts can also be toxic to methanotrophs (Schimel & Gullede 1998). Physical disturbances, such as tillage, can also cause the effective elimination of CH<sub>4</sub> oxidizers (Ojima *et al.* 1993) via a combination of compaction that reduces CH<sub>4</sub> diffusion and increased NH<sub>4</sub><sup>+</sup> availability (Hansen *et al.* 1993).

#### Step 2. Ecosystem-level differences in community physiology

*Methane Production.* A good example of ecosystem-scale differences in methanogen communities is from Valentine *et al.* (1993), who showed that the pH sensitivity of CH<sub>4</sub> production differed among Canadian peat soils. In the common neutral peats, methanogenesis was acid sensitive. In acid, 'black hole' sites, however, CH<sub>4</sub> production was enhanced by low pH, indicating the organisms were acid adapted. It is not clear what component(s) of the communities differed between these sites. Methanogens rely on other microbial groups to carry out depolymerization and fermentation that provide substrate (Valentine *et al.* 1993). Hence, the different pH optima of CH<sub>4</sub> production could result from community differences at any step.

Other evidence for differences in microbial community structure affecting CH<sub>4</sub> production comes from the temperature response of CH<sub>4</sub> production in different ecosystems. The Q<sub>10</sub> for methanogenesis varies widely, with estimates ranging from less than 2 to greater than 20 (Svensson 1984; Dunfield *et al.* 1993). As discussed earlier for CO<sub>2</sub>, different temperature responses suggest different communities either among the methanogens or fermenters. As different methanogenic populations (acetate vs. H<sub>2</sub> users) appear to dominate at different temperatures

(Schulz & Conrad 1996), differing distributions of these populations could produce different  $Q_{10}$  responses across ecosystems. Identifying the critical components of the community that control  $CH_4$  production remains a challenge.

*Methane consumption.* Perhaps the clearest demonstration of ecosystem-level influences of microbial community structure on trace gas dynamics comes from work on atmospheric  $CH_4$  consumption. This is probably because this is the 'narrowest' physiology of all the trace gas processes. First, most known methanotrophs, the 'common methanotrophs' (Conrad 1995), have low affinity for  $CH_4$ , with  $K_s$  (half-saturation constant for uptake) values for  $CH_4$  of greater than  $1 \mu M$  (Bedard & Knowles 1989; Lidstrom 1996). Upland  $CH_4$  oxidizers, however, typically have  $K_s$  values in the range of 10–100 nM (Conrad 1996; Gullede & Schimel, unpubl. data). This large difference in kinetic constants indicates physiological differences between wetland and upland methanotrophs. The range of kinetic constants observed among upland  $CH_4$  oxidizers may also indicate population differences within that group.

A second example of ecosystem-scale differences in  $CH_4$  oxidizer physiology was shown by Steudler *et al.* (1996) who measured the ratio of the rates of  $^{14}CH_4$  and  $^{14}CO$  oxidation in temperate forests and agricultural fields. A high  $CH_4/CO$  ratio was assumed to indicate that methanotrophs were the active population, while a low ratio would imply nitrifiers (Jones & Morita 1983). Steudler *et al.* (1996) found that across several ecosystems (pine, hardwood, and agricultural fields), soil horizons (organic and mineral), and fertilization treatments, the ratios varied from 0.446 to 0.0003. They concluded that disturbance (fertilization) and soil characteristics altered the active members of the  $CH_4$  oxidizing community, and that this altered ecosystem-level process dynamics, as flux rates correlated with  $CH_4/CO$  oxidation ratios.

A third example of community influence is  $NH_4^+$  inhibition of  $CH_4$  consumption, a commonly observed phenomenon (King 1997). There is, however, no uniform pattern of N inhibition *in situ* as would be expected from a single physiological mechanism. In fact, there are at least three inhibition patterns. In some sites, inhibition is immediate, occurring within hours or days (Nesbit & Breitenbeck 1992; Adamsen & King 1993; Gullede *et al.* 1997). In other sites, inhibition is delayed, taking several years to reach its maximum (Castro *et al.* 1995; Gullede *et al.* 1997). In still other sites,  $CH_4$  consumption is insensitive to  $NH_4^+$  (King 1992; Gullede *et al.* 1997). An Alaskan white spruce stand never showed inhibition in either the lab or field even after 5 years of fertilization, while a nearby birch stand showed delayed inhibition: no inhibition occurred in lab studies or short-term field

studies, but > 70% inhibition occurred after several years of field fertilization (Gullede *et al.* 1997). Differences in N-cycling (e.g. N-immobilization that could mask the  $CH_4$  oxidizers from  $NH_4^+$ ) could not explain the different inhibition patterns. Methane oxidizers in the two soils, however, were physiologically distinct (Table 1), providing a possible explanation for the different responses to  $NH_4^+$ . The relevant conclusion for global trace gas dynamics is that the response of  $CH_4$  oxidation to N inputs, and possibly other changes, will vary from system to system depending on the nature of the methane oxidizer population.

### Nitrogen gases

Nitrous oxide ( $N_2O$ ) and nitric oxide (NO) are produced in soil by both nitrification and denitrification, although under different conditions. While much work has been done on distinguishing between these processes as sources of N gases (McKenney & Drury 1997; Smith 1997), less has been done on how community composition within functional groups controls gas fluxes. Most N-gas production via nitrification is by autotrophic  $NH_3$  oxidizers (Tortoso & Hutchinson 1990). These are a physiologically narrow and phylogenetically coherent group of bacteria (Teske *et al.* 1994). NO and  $N_2O$  are both minor side products of nitrification (rarely exceeding 1–2% of the total  $NH_4^+$  oxidized, Firestone & Davidson 1989). Nitrifier communities vary among ecosystems as indicated by temperature sensitivity (Stark 1996) and kinetics (e.g.  $K_m$  for  $NH_4^+$ ; Stark and Firestone 1996), but little work has been done showing differences in nitrifiers affecting N-gas production.

Denitrification, on the other hand, is widely distributed taxonomically, including, for example, both the gram negative and gram positive bacteria. Even so, the physiology of denitrification is relatively narrow. The enzymes in the denitrification pathway are highly conserved, even among phylogenetically distant denitrifiers (Ye *et al.* 1993). Also, process control is fairly consistent among denitrifiers. Denitrification almost universally occurs in organisms that are primarily aerobic respirers, but which have the ability to shift to denitrification when  $O_2$  is limiting. However, there are differences in the specific control of the induction and activity of the enzymes in the denitrification pathway that could produce system level differences in activity under different conditions (Ferguson 1994). Cavigelli & Robertson (1996) suggested that differences in denitrifier communities in agroecosystems produced different  $N_2O$  production rates, under experimental conditions where resources were either nonlimiting or tightly controlled.

**Table 1** Physiological Characteristics of CH<sub>4</sub> oxidizers in two taiga soils. From Gullede *et al.* (1997)

Site	Pattern of Inhibition due to NH <sub>4</sub> <sup>+</sup> additions	Inhibited by K <sub>2</sub> SO <sub>4</sub> additions	Showed apparent growth on 1.7 ppm CH <sub>4</sub>	Maximum CH <sub>4</sub> oxidation rate* (pmol g <sup>-1</sup> h <sup>-1</sup> )
Birch	Delayed	No	Yes	7.92
White spruce	None	Yes	No	75.7

\* Initial CH<sub>4</sub> concentration was about 1.8 ppm.

### Intermediate conclusions— the need to develop microbial community studies in an ecosystem framework

Based on the studies discussed above, we believe that microbial community structure *can* influence trace gas dynamics in terrestrial ecosystems. We have shown that there are cases where differences in ecosystem function appear to result from differences in the microbial communities. Thus, in some cases it is likely that global change will alter the structure of those communities in ways that may have unpredicted effects on their biogeochemical processes. Being able to predict the nature of those changes requires a better understanding of the community dynamics of the critical organisms. Developing microbial community studies directed at making those predictions would provide a valuable link between microbial ecology and global change science, while advancing both fields significantly. In the next section of this paper, we discuss an approach to developing studies that would effectively make that linkage, and in the process satisfy steps 3–6 for understanding microbial community control over ecosystem trace gas dynamics.

Three things are critical in studying the role of microbial community structure in global change. First, studies must be done in a way that effectively relates microbial community structure and activity to the structure and functioning of the whole ecosystem. Merely identifying an organism capable of a particular process without determining its role, if any, in ecosystem function has limited value for this purpose. Second, effective tools must be developed for identifying the *active* members of the microbial community, rather than for merely detecting the occurrence of particular organisms. To do so, we must be able to detect active but uncharacterized microbial populations. Finally, hypotheses regarding the distribution of organisms *in situ* must be quantitative. That is, we must employ statistical analyses to demonstrate linkages between microbial populations and ecosystem-level process dynamics, and also for detecting significant changes in microbial community composition.

### Approaches to analysing communities

For an initial characterization of soil microbial communities, broad-scale techniques can be used to detect gross changes in community structure and to identify shifts in major groups of microorganisms in response to a change in environment or land-use. Cell membrane lipid fatty acids (phospholipid fatty acids – PLFA) are useful biomarkers as they have great diversity coupled with biological specificity. In a soil community, PLFAs can distinguish broad taxonomic groups, such as gram-negative and -positive bacteria, actinomycetes, and fungi, and may also be used to 'fingerprint' the microbial community in order to monitor changes in composition (Frostegård *et al.* 1993, Bardgett *et al.* 1996). Some narrower taxa, such as methanotrophic bacteria, also may be distinguished by PLFAs (Bowman *et al.* 1991). For bacterial populations, another approach for detecting gross changes in community dynamics is substrate-use patterns and kinetics (e.g. Bossio & Scow 1995; Lindstrom *et al.* 1997). Although substrate-use patterns are difficult to relate to *in situ* bacterial community function, the approach offers a sensitive detector of changes in community behaviour in response to altered environmental conditions (Lindstrom *et al.* 1997).

Although broad-scale measurements are useful for examining gross differences in microbial community structure, they offer little in connecting specific microbial populations with ecosystem dynamics. Analysing the active members of the microbial community is a challenge, though modern molecular techniques such as 16S rRNA analysis and quantitative gene probe hybridization provide tools for addressing this challenge (e.g. Amann *et al.* 1995; Hugenholtz & Pace 1996; Lidstrom 1996). Applying these techniques to soils has been difficult, but substantial progress is being made (e.g. Jacobsen 1995; Borneman *et al.* 1996; Felske *et al.* 1996; Zhou *et al.* 1996). The key to the power of molecular techniques is that organisms can be identified without cultivating them or even having previous knowledge of them (Amann *et al.* 1995). Nucleic acids (both DNA and RNA) can be extracted from soil and tested against oligonucleotide

probes of varying specificity. For instance, probes for the DNA sequences for particular enzymes may identify the occurrence of organisms based on their function. Similarly, probes for 16S rRNA sequences can be used to study the distribution of uncultured organisms based on their phylogeny; such probes range in specificity from those that select for all *Bacteria*, *Archaea*, or *Eukarya*, to those that select for individual species (Woese 1987). Hence, genetic techniques can be used to examine microbial communities at multiple scales of resolution, and offer the sensitivity to detect and track individual populations, even when they have not been identified previously.

Merely because an organism is detectable, however, does not indicate that it is active. Equally, failing to detect a specific organism does not prove that it is not present and active (Hovanec & DeLong 1996). Many more organisms are detectable by molecular methods than by traditional microbiological techniques (Barbieri *et al.* 1996; Borneman *et al.* 1996), but an organism may still lack sufficient genetic similarity to known organisms to hybridize to existing probes (Amann *et al.* 1995). Considering that only a small proportion of the microorganisms in most natural communities have been isolated, it should not be surprising to find many novel organisms in soils (Liesack & Stackebrandt 1992; Borneman *et al.* 1996). Molecular techniques have greatly enhanced our ability to detect and identify soil microorganisms, but they have been used primarily to inventory species diversity or to survey the distribution of previously identified organisms. They have not been used routinely to determine which organisms are active *in situ*.

The crucial task then, is to determine which organisms drive ecosystem processes. We need approaches that provide sensitive detection of populations without requiring previous knowledge of them, combined with the ability to determine the physiological activity of those populations. Linking active microbial populations to ecosystem process dynamics will require using a combination of molecular and biogeochemical approaches. For instance, it may be possible to manipulate microbial activity by altering substrate supply, or to exploit natural gradients within or among ecosystems to identify nucleic acid sequences associated with the populations carrying out specific processes. Examples of this type of approach are rare, but at least two have been published recently (Holmes *et al.* 1995b; Teske *et al.* 1996). We will suggest some strategies by which microbial communities can be characterized with respect to ecosystem function; these are not intended as a technical primer or as an exhaustive suite of approaches.

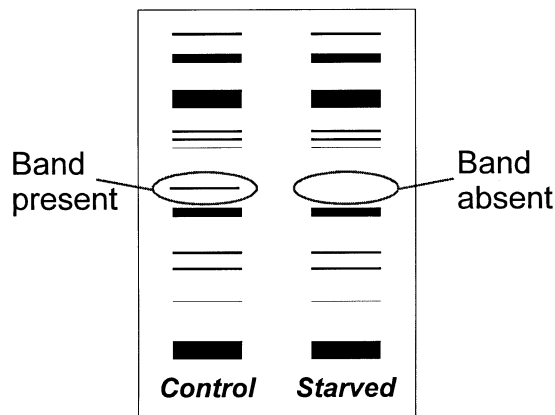
One way to identify an active population is to use quantitative hybridization techniques (Amann *et al.* 1995) with gene probes for known organisms to determine how they respond to ecological gradients or specific

manipulations (Pace 1996; Teske *et al.* 1996). We can manipulate populations by, for example, using specific inhibitors (Oremland & Capone 1988; Roy & Knowles 1995) or altering substrate availability (Holmes *et al.* 1995b; Schnell & King 1995; Gullede *et al.* 1997). We can measure changes in microbial activity by using physiological assays, such as CH<sub>4</sub> oxidation capacity, nitrification potential, or soil respiration (Davidson & Schimel 1995). If changes in the population of the 'suspect' organism, as indicated by gene probes, correspond to changes in the process, that is indication that the organism is an active member of the community. If the physiology of the known organism (e.g. K<sub>s</sub> for substrate uptake) also corresponds to the physiology of the total community that would strengthen the conclusion (Lidstrom 1996). However, even when such correlations are observed, it remains possible that other unidentified populations are also active. Correlations between known organisms and *in situ* process dynamics may be rare, as unknown populations may often dominate (Lidstrom 1996). It is crucial that complementary approaches be developed that do not rely on previously characterized organisms.

One approach that does not require previous knowledge of an organism is to compare an inventory of 16S rRNA sequences [developed using community analysis techniques, such as clone libraries—Holmes *et al.* 1995b; or denaturing gradient gel electrophoresis (DGGE)—Teske *et al.* 1996; Ferris *et al.* 1996] in a manipulated sample to those in an untreated sample. Assuming the manipulation affected only the target population(s), differences in the rRNA inventories would correspond to the active populations (Fig. 4). This could be confirmed by isolating rDNA suspected of belonging to the active populations, developing specific probes, and using those to study the population *in situ* as discussed in the previous paragraph. Additionally, the rDNA sequence may be used to determine the phylogenetic relationship of the identified organisms to other known organisms. Holmes *et al.* (1995b) used a similar strategy to examine methanotroph populations manipulated by CH<sub>4</sub> enrichment in seawater samples.

A similar approach to identify active members of a community is to analyse the ratio between the rRNA of a specific population, and the DNA that encodes the rRNA (rDNA; Teske *et al.* 1996). Active cells tend to possess higher numbers of ribosomes and larger ratios of rRNA to rDNA than inactive populations (Kemp 1995). Populations whose rRNA/rDNA ratios change in synchrony with the process of interest may therefore be actively driving that process. The rDNA sequences may be used for identifying the organisms and for designing population-specific probes for quantitative hybridization. Teske *et al.* (1996) used this approach to examine the

### Use of physiological manipulations to find markers for active organisms



**Fig. 4** A hypothetical example of using DGGE analysis to identify the active organisms in a biogeochemical process. By comparing the rRNA extracted from a control sample and one which has been starved for substrate (e.g. CH<sub>4</sub>), it is possible to identify the rRNA associated with the active population of bacteria.

community structure and activity of SO<sub>4</sub><sup>=</sup> reducers along biogeochemical gradients in a stratified fjord.

#### *Linking microbial community structure to ecosystem function: Steps 4–6*

Using the above methodological background, we can develop a strategy for addressing steps 4–6 for understanding microbial controls over trace gas dynamics. The active community can be analysed across sites that show different physiological responses to environmental change, e.g. different pH, temperature, or N response. This establishes the relationships between phylogenetic and physiological diversity within key microbial groups. Comparing these relationships across ecosystems addresses step 4, determining how the different populations are distributed with geography and climate. Molecular studies have generally avoided such quantitative comparisons because of the labour involved in sample processing and the lack of quantitative precision in the techniques. These difficulties must be overcome in order to assess the role of microbial community structure in ecosystem function, particularly within the context of global change.

Addressing objective 5 requires the use of information gathered in previous steps combined with repeated experimentation to identify responses of various microbial populations to changing conditions. These studies can either apply manipulative approaches such as artificial soil warming (Peterjohn *et al.* 1993), fertilization (Stuedler *et al.* 1989; Gullede *et al.* 1997), other standard

approaches that have allowed us to develop mechanistic models of biogeochemical processes (Davidson & Schimel 1995), or they may involve gradient analysis to evaluate how communities change across a range of conditions (Teske *et al.* 1996; Ramsing *et al.* 1996). These studies will include standard approaches to analysing the physiology of trace gas processes (Davidson & Schimel 1995), but will focus on the population and community dynamics rather than just their physiology. These mechanistic studies with a community ecology focus will provide much of the data needed to develop predictive models that integrate microbial community dynamics with ecosystem-level process dynamics, thus accomplishing step 6.

#### **Possible scenarios of climate change effects on microbial communities.**

Despite the material we have presented, one could still raise the question: 'Even if we accept that variation in microbial communities can cause ecosystem level changes, and that we can determine how communities may change, how does that advance our ability to predict trace gas fluxes under conditions of altered climate and land use?' In this section, we develop several possible scenarios of how global change effects could alter soil microbial communities and through this, ecosystem-scale trace gas processes, and further, how study of these links might be of value to modelling trace gas fluxes.

The first example deals with changing precipitation and its effects on trace gas fluxes. Most, if not all, ecosystem models use simple response functions between a process and environmental conditions. Such models generally do not incorporate hysteresis. As a soil dries and then rewets, the models would predict the same process rate at a given soil moisture regardless of whether the soil has dried down or has been rewet. Yet, studies have shown that hysteresis can be important in controlling microbial processes and trace gas fluxes (Moore & Roulet 1993). The cause of hysteresis is probably usually due to changes in microbial communities.

Water table height is an important factor controlling CH<sub>4</sub> fluxes from wetlands, because it controls the extent of anaerobic-CH<sub>4</sub> producing soil and the amount of CH<sub>4</sub> consumed before it escapes. However, CH<sub>4</sub> fluxes can vary significantly depending on whether the water table is rising or falling. Moore & Roulet (1993) showed that fluxes could differ by more than 100-fold at a given water table height depending on whether the water table was rising or falling. It is likely that high emission rates when lowering the water table are due in part to the large populations of both methanogens and the fermenters that supply their substrate as well as the small populations of methanotrophs that develop while the soil is flooded; conversely, when the water table is low, fermentative and

methanogenic populations decline while methanotroph populations increase. Thus, raising the water table to a given height after a dry period results in much lower emission rates than lowering the water table to the same level. In areas where water table heights rise and fall seasonally, it is impossible to model CH<sub>4</sub> fluxes accurately without accounting for the hysteresis due to changing microbial populations. This may be one major reason why wetland CH<sub>4</sub> fluxes have proven so difficult to model (Moore & Roulet 1993). Microbial studies that allow us to predict how well the anaerobic populations survive in aerated conditions and how fast they regrow following the onset of flooding and anaerobiosis might prove valuable in developing more effective models of CH<sub>4</sub> fluxes under changing conditions. Similarly, understanding the effects of periodic anaerobiosis on methanotroph populations could also improve our ability to predict wetland CH<sub>4</sub> fluxes under changing moisture conditions. Most wetlands have seasonally varying water tables, and there are fairly extensive wetlands that are only seasonally flooded. The extent of water table fluctuation and seasonal flooding could vary extensively with climate change. Anthropogenic changes in land and water use, such as rice agriculture, dam building, and flood control, could also effect the extent and timing of flooding and CH<sub>4</sub> fluxes over large areas. Without a better understanding of microbial community dynamics, it may be impossible to predict effectively how CH<sub>4</sub> fluxes will respond to long-term environmental changes.

Hysteresis may also occur in CO<sub>2</sub> fluxes when unsaturated soils undergo drying and wetting cycles. As climate changes, much of the world will likely experience more evapotranspiration and soils will therefore dry more rapidly (Eatherall 1997). Thus the frequency and intensity of soil drying and rewetting events may well increase. A single drying/wetting event can kill up to 50% of the microbial community (Kieft *et al.* 1987). We know very little about which organisms survive drying/rewetting shocks or how fast they recover. Clein & Schimel (1994) suggested that populations of key litter degraders could be reduced for an extended period, yet some component of the microbial biomass is resistant to drying/rewetting stress (Bottner 1985). For litter, at least, we can hypothesize that in areas where episodic drying and rewetting become more severe, that populations of cellulolytic and lignolytic fungi could be reduced to the point where litter decomposition would decrease more than would be predicted by simply the changes in moisture. This would decrease CO<sub>2</sub> flux, at least during the seasons when episodic stresses occur. In areas with a distinct wet season, this might simply change the seasonal pattern of CO<sub>2</sub> flux, with a greater fraction of decomposition occurring during the wet season. But, in areas without a distinct wet season, reduced litter degrading populations could lead

to a greater litter build-up. Ultimately, this could result in greater C and nutrient sequestration and lower plant productivity. This could also increase fuel loads and cause more common or more intense fires, with impacts on trace gas fluxes (Hao & Ward 1993; Levine *et al.* 1996). Research on the dynamics of critical populations and their responses to stress using the techniques we have discussed will allow us to test these hypotheses and if supported, allow us to integrate the knowledge into biogeochemical models.

Another possible scenario has to do with CH<sub>4</sub> oxidation and the strength of the soil sink for atmospheric CH<sub>4</sub>. As mentioned, this is not a very large sink in global terms, but changes in the soil sink could greatly affect atmospheric CH<sub>4</sub> concentrations and the rate of increase (Ojima *et al.* 1993). A decreased soil sink could cause more of the atmospheric OH radicals to be used in oxidizing CH<sub>4</sub>, while they would otherwise serve to oxidize other atmospheric pollutants such as hydrocarbons, carbon monoxide, NO<sub>x</sub> and SO<sub>x</sub>. Thus, relatively small changes in the soil CH<sub>4</sub> sink could have relatively large impacts on the atmosphere (Ojima *et al.* 1993).

Building large-scale models of how the soil CH<sub>4</sub> sink will change with climate, N-availability, and land use, requires being able to predict the response of soil CH<sub>4</sub> oxidizers to these changing conditions. Research to date has shown that CH<sub>4</sub> oxidation in soil can vary substantially in its response to salinity, N-availability, and possibly vegetation (Gulledge *et al.* 1997). Moreover, the recovery of a CH<sub>4</sub> oxidizing population after even a single stress event can take years (Mosier *et al.* 1991). Thus, predictions of CH<sub>4</sub> consumption using models that assume a constant sensitivity and no postdisturbance lags may be seriously in error. Given the apparent slow postdisturbance recovery of populations, CH<sub>4</sub> consumption may be more sensitive to global changes than would be otherwise predicted. We do not understand how factors such as NH<sub>4</sub><sup>+</sup> or salt sensitivity are distributed among the native CH<sub>4</sub> oxidizer populations, yet it is clear that these factors vary among ecosystems (Gulledge *et al.* 1997). In the perspective of global change, the ultimate goal of research on CH<sub>4</sub> oxidizer population dynamics would be to determine how the sensitivity of CH<sub>4</sub> oxidizers to different environmental stresses are distributed relative to the natural occurrence of those stresses, how fast populations recover from stress-induced crashes, and how fast populations will adapt to changing environmental conditions. As discussed previously, achieving these objectives requires that we identify the active populations and determine their immediate and ultimate responses to environmental change.

Research on the population dynamics of microbes driving trace gas fluxes will have two valuable outcomes. One will be increasing our knowledge of the organisms

that drive the earth system, as we are aware of only a very small percentage of the microorganisms active in natural ecosystems. Such work will advance our basic knowledge of microbial ecology and ecosystem science, and is worth pursuing on its own merits (Pace 1996). Second is the direct application to global change science. A primary goal in global modelling is developing the feedbacks between the atmosphere and biosphere (Bretherton *et al.* 1992). Many of the feedbacks may occur through changing populations of plants, animals, and microbes. Understanding the biological mechanisms of the feedbacks will allow us to develop more reliable models of atmospheric trace gas dynamics under altered global conditions. In this larger context, the ultimate success of microbial community studies would be to condense years of intense study into a single parameter or response surface in a model that never explicitly includes microbial populations. That would indicate that we understand the population dynamics well enough that we can 'model past' them: from the drivers of population changes directly to the effects of them. This approach of simplifying and condensing the complex into a few simple parameters has been the pathway for effectively incorporating microbial physiology into ecosystem-scale models. It was only through solid physiological study, rather than empirical studies, that we have been able to develop models that incorporate essential controls and process dynamics. This same approach will allow us to integrate microbial population dynamics into global change models and studies. In this case, however, we must move beyond process physiology to the individual populations that drive ecosystem processes and learn how these populations respond to a changing environment.

## Conclusions

We draw three major conclusions. First, there is evidence that differences in microbial community structure affect trace gas dynamics at the ecosystem scale. These differences manifest either in different process kinetics or in altered control mechanisms on trace gas fluxes. Second, the tools exist to analyse the structure of the active microbial community and to relate specific microbial populations to their biogeochemical activities. Third studies linking community and process ecology should be developed in a way that tightly integrates modern microbial ecology techniques with the types of manipulative approaches that have been critical to developing a mechanistic understanding of trace gas biogeochemistry.

The appropriate studies on microbial communities have challenges of methodology, logistics, and interpretation. We contend, however, that combining traditional microbiological and biogeochemical approaches with

modern molecular techniques affords an unprecedented opportunity to link ecosystem-level process dynamics directly to microbial community composition and function. Furthermore, we contend that efforts to make such linkages represent a logical next step in the advancement of both ecosystem science and microbial ecology relative to global change. The practical need to understand the role of soil biota in the cycling of trace gases provides both the ecological framework and the scientific and social impetus needed for initiation and development of this important area of research.

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