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ORIGINAL ARTICLE

Agriculture's impact on microbial diversity and associated fluxes of carbon dioxide and methane

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Agriculture has marked impacts on the production of carbon dioxide (CO_2) and consumption of methane (CH_4) by microbial communities in upland soils—Earth's largest biological sink for atmospheric CH_4. To determine whether the diversity of microbes that catalyze the flux of these greenhouse gases is related to the magnitude and stability of these ecosystem-level processes, we conducted molecular surveys of CH_4-oxidizing bacteria (methanotrophs) and total bacterial diversity across a range of land uses and measured the *in situ* flux of CH_4 and CO_2 at a site in the upper United States Midwest. Conversion of native lands to row-crop agriculture led to a sevenfold reduction in CH_4 consumption and a proportionate decrease in methanotroph diversity. Sites with the greatest stability in CH_4 consumption harbored the most methanotroph diversity. In fields abandoned from agriculture, the rate of CH_4 consumption increased with time along with the diversity of methanotrophs. Conversely, estimates of total bacterial diversity in soil were not related to the rate or stability of CO_2 emission. These combined results are consistent with the expectation that microbial diversity is a better predictor of the magnitude and stability of processes catalyzed by organisms with highly specialized metabolisms, like CH_4 oxidation, as compared with processes driven by widely distributed metabolic processes, like CO_2 production in heterotrophs. The data also suggest that managing lands to conserve or restore methanotroph diversity could mitigate the atmospheric concentrations of this potent greenhouse gas.
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Introduction

Carbon dioxide (CO_2) and methane (CH_4) are currently responsible for about 80% of the radiative forcing from long-lived greenhouse gases in Earth's atmosphere (Forster *et al.*, 2007). The atmospheric concentrations of both gases are higher than they have been over the past 650 000 years and continue to rise (IPCC, 2007; Rigby *et al.*, 2008). Land-use change, especially deforestation and row-crop agriculture (Schlesinger and Andrews, 2000; Smith *et al.*, 2000), has an important role in determining the rate of these fluxes and also changes the structure of microbial communities in soil (Borneman and Triplett, 1997; Smith *et al.*, 2000; Zhou *et al.*, 2008).

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The linkage between altered trace gas emissions and microbial community structure in soil may be more than associative—contemporary models in ecology suggest a positive relationship between species diversity and the magnitude and stability of ecosystem processes catalyzed by those species (Tilman *et al.*, 2001; Symstad *et al.*, 2003; Fargione *et al.*, 2007; Ives and Carpenter, 2007). Should this relationship also hold for the soil microbial communities responsible for trace gas production and consumption, opportunities may emerge for mitigating emissions. For microbes in soil, however, the relationship between species diversity and the magnitude and stability of trace gas fluxes has rarely been tested. Although evidence to date suggests that such a relationship is unlikely for decomposition and other soil processes broadly catalyzed by many microbes (Schimel, 1995; Groffman and Bohlen, 1999), diversity may matter substantially for processes catalyzed by microbial groups more specialized and depauperate (Cavigelli and Robertson, 2000).

We tested the hypothesis that the magnitude and stability of CH_4 consumption in well-drained soils, a process catalyzed by relatively few bacteria, is

positively correlated with the diversity of methanotrophs present. We also tested the corollary hypothesis that the magnitude and stability of CO_2 production, a process carried out by all heterotrophic bacteria in soil, is not related to the diversity of bacteria present.

We measured *in situ* fluxes of CO_2 and CH_4 and assessed microbial diversity across five different land-use intensities at the Kellogg Biological Station Long-Term Ecological Research (KBS LTER) site in southwest Michigan. KBS LTER, with replicated plots of the same soil series (Crum and Collins, 1995) that differ in land use, represents an ideal system for determining how land use impacts microbial communities and the greenhouse gas fluxes they control. Past studies have typically investigated either gas flux or the microbial community at analogous sites, but rarely has the function mediated by the microbes and the microbial community been investigated simultaneously across a land-use gradient. Our intent was not to unravel the root causes of variation in either community structure or greenhouse gas flux, but to show an association that, if directional, could suggest insights into long-term management options for the microbial community and their gas fluxes. To our knowledge, this is the first study to correlate methanotroph and bacterial diversity to both the stability and magnitude of the associated *in situ* greenhouse gas fluxes across a successional land-use gradient, and to use the results to gain insights into management options for abatement of atmospheric greenhouse gas increases.

Materials and methods

Gas-flux measurements

In situ rates of CH_4 consumption and CO_2 production were measured using closed-cover flux chambers (Robertson *et al.*, 2000) across a gradient of land uses at the KBS LTER site located in Hickory Corners, Michigan. Land-use treatments range from conventional row-crop agriculture (AG) (wheat–corn–soybean rotation, chisel plowed, conventional fertilizer and pesticide inputs) to intact, late-successional deciduous forest never converted to agriculture (DF), as well as three types of replicated successional communities: an early successional community abandoned in 1989 (ES), an unmanaged mid-successional community abandoned in the 1950s (SF), and a never-plowed never-planted community managed as an annually mowed grassland following forest clearing in 1960 (MG). All five treatments are replicated ($n = 3$ one hectare plots) on the same soil series with similar textures (Kalamazoo/Oshtemo, and are well-drained Typic hapludalfs (fine or coarse loamy, mixed)) (Crum and Collins, 1995)—but with land-use associated differences in net primary production, soil organic matter (Robertson *et al.*, 2000), aggregation (Grandy and Robertson, 2007), and derived properties, such as moisture content and diffusion. Additional site information is available at http://lter.kbs.msu.edu. Gas-flux measurements were typically made twice monthly between March and December: detailed protocols, field logs and data are available at http://lter.kbs.msu.edu/datasets. Gas-flux data reported in this study are from 1993–2008 (KBS013-001), and soil moisture and soil temperature measures associated with the gas-flux data from 2003–2008 (KBS013_003 and KBS013_004) were downloaded on 2 March 2010. Data for treatments MG and SF are available from 1993–1997 and 2008. Averages incorporate all monthly measurements for the reported time period.

Assessment of total bacterial diversity

Five soil cores (2.5×10 cm) were collected and pooled from each of two replicate plots in December 2008 representing the five land uses. Plant litter and roots were removed by sieving (4 mm sieve), which also served to homogenize the cores. The composited samples were flash frozen in liquid N_2, transported to the laboratory on ice and stored at $-80\,^{\circ}C$. In all, 5-g subsamples were ground in sterile sand and liquid N_2 with a mortar and pestle. Microbes were lysed enzymatically and chemically, followed by isolation of the released DNA using acetyl trimethylammonium bromide-based method (Zhou *et al.*, 1996) and further purification on cesium chloride gradients (Sambrook and Russell, 2001). Sequence tags for the V6 region of the 16S rRNA-encoding gene were generated (Sogin *et al.*, 2006) and sequenced using Life Science's 454 GS-FLX pyrosequencing chemistry (Huse *et al.*, 2008) at the Woods Hole Marine Biological Laboratory Bay Paul Center. An average of 15 800 sequence tags were generated for each sample. Membership in an operational taxonomic unit (OTU) was defined as sequences sharing 97% nucleotide identity using the VAMPS MBL Bay Paul Center pipeline (Huse *et al.*, 2008).

Methanotroph census

The diversity of methanotrophs across the KBS LTER landscapes was assessed via PCR amplification of the β-subunit of particulate methane monooxygenase (*pmoA*). Template DNA for the amplification reactions was purified with the MoBio (Carlsbad, CA, USA) PowerSoil DNA Isolation Kit from 0.25 g subsamples from each of 75 soil cores collected in June 2006: 5 cores from each of 3 replicates of the 5 land-use treatments. *pmoA* was amplified from 45 ng of template DNA with primers A189 (5′-GGNG ACTGGGACTTCTGG-3′) and A682 (5′-GAASGCNG AGAAGAASGC-3′; labeled with 6-carboxyfluorescein) (Holmes *et al.*, 1995). The products of technical replicates of PCR reactions were pooled to minimize amplification bias, then purified and

digested sequentially with three restriction endonucleases for terminal restriction fragment length polymorphisms analysis. See Supplementary Information for detailed protocols.

Clone libraries of *pmoA* amplicons were created from a selection of soil samples and amplification conditions (Supplementary Table S1) to provide a molecular phylogeny of methanotrophs. Clone libraries were sampled until rarefaction curves were asymptotic (Supplementary Figure S5). Sequences from clone libraries were binned using DOTUR (Schloss and Handelsman, 2005), if they shared 94% average nucleotide sequence similarity—a species-level designation for functional genes (Konstantinidis *et al.*, 2006). Representative *pmoA* sequences were deposited in GenBank under accession numbers FJ529724–FJ529808, and GQ219582 and GQ219583.

Statistical analysis

The effect of land use on CH_4 consumption and methanotroph richness was explored through *t*-tests, and a one-way multivariate analysis of variance (SAS Institute Inc., Cary, NC, USA). Linear regressions were used to relate mean greenhouse gas flux and species diversity, as well as the recovery of mean CH_4 consumption and mean methanotroph diversity following cessation of agriculture. Multiple regression analysis was used to assess the relative contribution of temperature and soil moisture to gas flux. The Sørenson index was calculated for each pairwise comparison of methanotroph communities in KBS LTER treatments and plotted using two-dimensional non-metric multidimensional scaling (Hammer *et al.*, 2001). A one-way analysis of similarities was used to assess the significance of differences between communities (Hammer *et al.*, 2001).

Results

Greenhouse gas flux and abiotic regulators

Rates of CH_4 consumption varied significantly with land use: the highest average annual rate of consumption was measured in the deciduous forest. Mid-successional fields, either abandoned from agriculture 50 years ago (SF) or never-tilled sites managed as grasslands (MG), consumed CH_4 at a significantly decreased rate as compared with the forest (DF), but significantly higher than early successional plots (ES) or agricultural plots (AG) (*t*-tests, $P<0.01$). The lowest rates of CH_4 consumption were consistently recorded in plots currently managed for row-crop agriculture (Figure 1a). There was approximately a sevenfold difference between the average annual rate of CH_4 consumption in the deciduous forest and the conventional agriculture plots (7.93 and 1.02 g CH_4-C ha^{-1} day^{-1}, respectively).

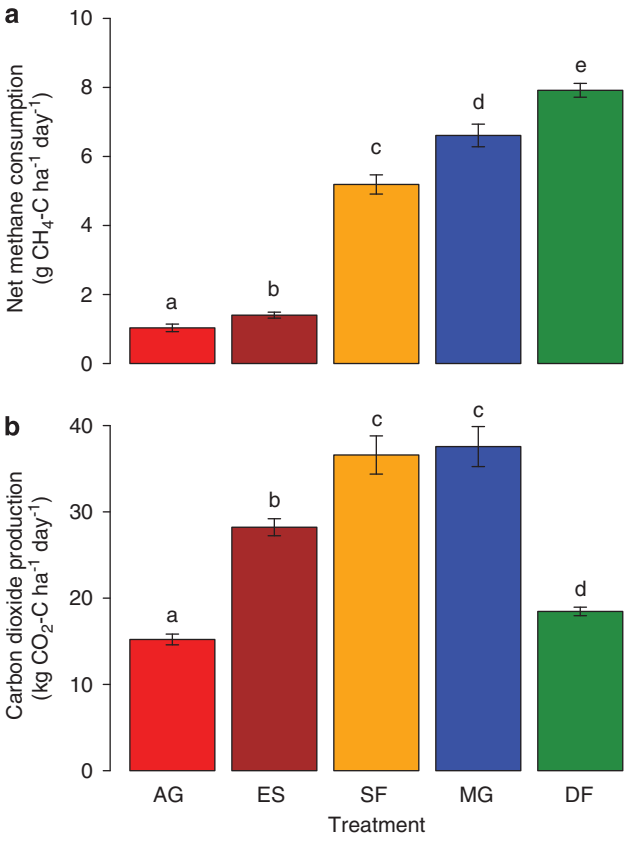

Figure 1 The effect of land use on CH_4 consumption (**a**) and CO_2 production (**b**) at the KBS LTER Site. Mean flux values are on the basis of all measurements available between 1993 and 2008. Land-use treatments are as follows: agricultural management of historically tilled lands (AG), early successional fields abandoned from agriculture in 1989 (ES), successional forests abandoned from agriculture in the 1950s (SF), managed grasslands on never tilled soil (MG), and deciduous forests (DF). Different letters represent significant differences ($P<0.05$) between treatments. Error bars represent standard errors.

CO_2 flux from soil also varied with land use. Soils in the early and mid-successional sites emitted 80% more CO_2 than the agricultural site, whereas the rate in the deciduous forest was 18% higher than the agricultural site. The highest rates of CO_2 emission and CH_4 consumption were recorded during the summer months for all treatments (Supplementary Figure S1); seasonal flux patterns were similar for all land uses. Soil temperature and moisture, two master regulators of biological processes in soil, explained portions of both fluxes, but were 10 times more effective in explaining the efflux of CO_2 ($r^2=0.12$, $P<0.001$) than rates of CH_4 consumption ($r^2=0.01$, $P=0.06$).

Methanotroph diversity and CH_4 consumption

To relate methanotroph diversity to annual averages of CH_4 consumption across the gradient of land uses at the KBS LTER, a method employing terminal restriction fragment length polymorphisms was developed to capture all methanotrophs present,

rather than methods that would assess metabolically active populations of methanotrophs at a particular time. The *pmoA* gene was targeted for this assay because it encodes a subunit of particulate methane monooxygenase—the first enzyme in the pathway of CH_4 oxidation and the defining enzyme of aerobic methanotrophs, except for *Methylocella* species (McDonald *et al.*, 2008; Rahman *et al.*, 2010). The terminal restriction fragment length polymorphism assay distinguished 11 OTUs that were confirmed as *pmoA* gene fragments both through sequence analysis and comigration of terminal restriction fragments with those from cloned controls. These 11 OTUs encompassed all of the major clades of methanotrophs defined by the *pmoA* gene sequences from clone libraries from KBS LTER (Supplementary Figure S2).

Methanotroph diversity, like the rates of CH_4 consumption, also varied significantly with land use ($P<0.05$; analysis of variance) (Supplementary Table S2B). The fewest methanotroph OTUs and lowest rates of CH_4 consumption were found in the agricultural plots, whereas the deciduous forest harbored the greatest number of methanotroph OTUs and the highest rates of CH_4 consumption. Modeling treatment effects on rates of CH_4 consumption while excluding methanotroph diversity as a covariate yielded reduced experimental error and indicated that methanotroph richness influences the rate of CH_4 consumption (Supplementary Table S2C).

There was a strong positive correlation between average annual rates of CH_4 consumption and the number of *pmoA* OTUs across the KBS LTER landscapes ($r^2=0.52$, $P<0.004$) (Figure 2a). Regression of annual rates of CH_4 consumption from 1993–2008 versus *pmoA* diversity yields a nearly identical relationship ($r^2=0.55$, $P=0.002$), as does summer rates of CH_4 consumption ($r^2=0.52$, $P=0.003$).

To further explore methanotroph diversity and search for new *pmoA* genes, 12 *pmoA* clone libraries were created from DF and AG samples with additional amplification conditions (Supplementary Table S1, SI text). All libraries were sampled until rarefaction curves were asymptotic (Supplementary Figure S5), in an effort to capture the full sequence diversity in each library. Although altered amplification conditions captured some additional *pmoA* genes, diversity was always greater in the forested plots as compared with the agricultural treatment (Supplementary Figure S3). Clone libraries revealed a new clade of methanotrophs, KBS1 (Supplementary Figures S2, S3), in addition to those previously identified in soils (Horz *et al.*, 2005; Knief *et al.*, 2005; Lau *et al.*, 2007). Using a species definition of 94% identity in functional genes (Konstantinidis *et al.*, 2006), 24 methanotrophic species were identified in cumulative libraries from the forested plots, but only 7 from the agricultural plots. As has been described previously, some subunits from ammonia monooxygenase genes (*amoA*) also amplified with the *pmoA* primers (Holmes *et al.*, 1995). The *amoA* clones were recognized by the presence of diagnostic amino acids (Horz *et al.*, 2005; Stoecker *et al.*, 2006) and their clustering in phylogenetic trees (Supplementary Figure S3). They were excluded from methanotroph diversity estimates.

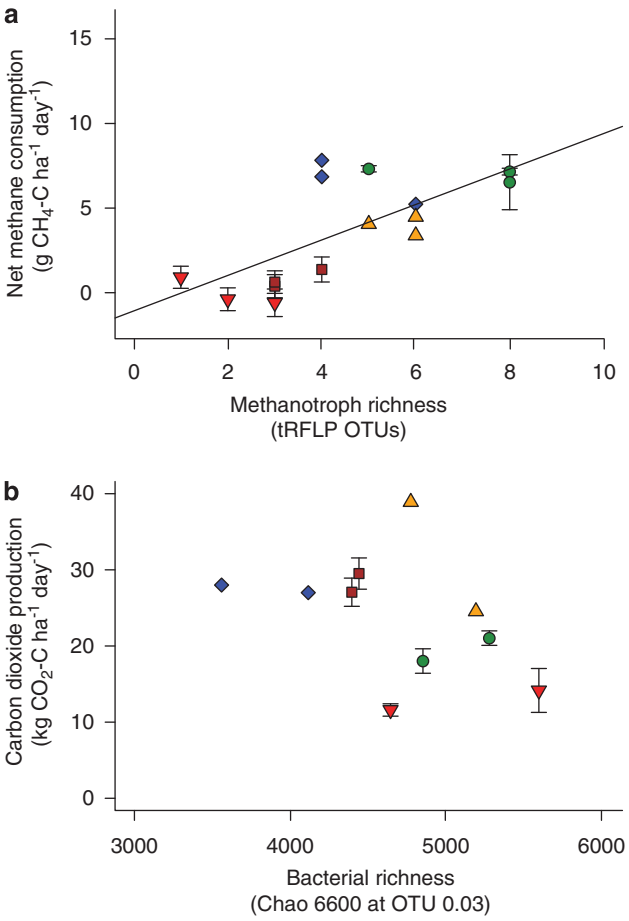

Figure 2 Relationship between average annual CH_4 consumption and methanotroph richness (**a**: $r^2=0.52$, $P<0.004$), and between average annual CO_2 production and bacterial richness (**b**: $r^2=0.17$, $P=0.22$) across land-use treatments at the KBS LTER. Gas flux and diversity data were collected between 2006 and 2008. Methanotroph richness is represented by OTUs that are on the basis of peaks in the terminal restriction fragment length polymorphism analysis that have been confirmed as originating from *pmoA* genes. Detailed descriptions of symbols can be found in the legend to Figure 1: ▼=AG, ■=ES, ▲=SF, ◆=MG, ●=DF. Error bars represent standard errors.

Bacterial diversity and CO_2 flux

We estimated bacterial diversity through analysis of 454 pyrosequencing of the V6 region of the 16S rRNA encoding gene (Sogin *et al.*, 2006) and clustering reads at 97% identity (Huse *et al.*, 2007). An average of 15 800 sequence tags was analyzed for each of the replicated samples. Rarefaction curves (Supplementary Figure S6) suggest that we did not capture the full sequence diversity, and so we used the Chao 1 estimator of

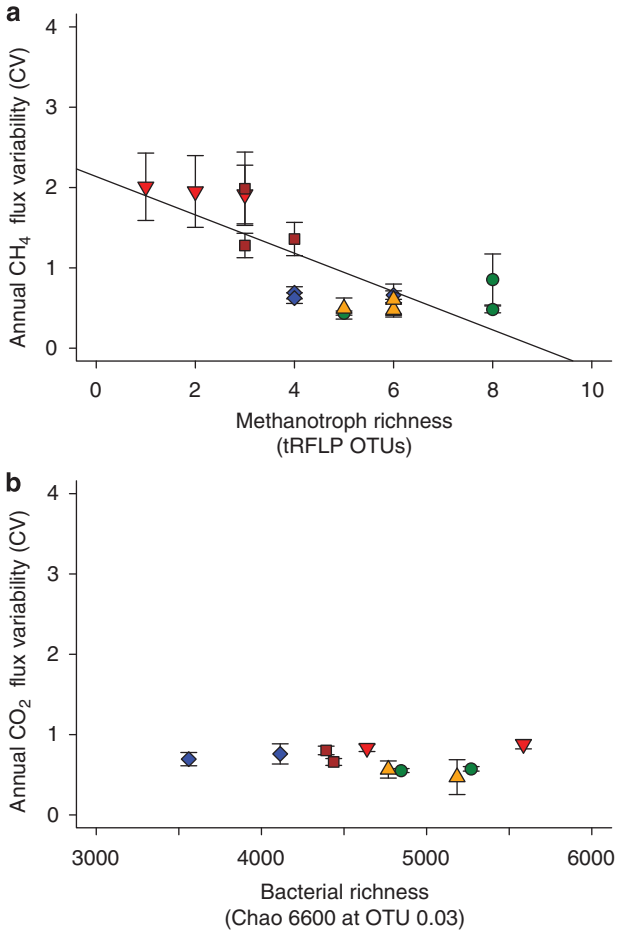

Figure 3 Variability in the flux of CH_4 in relation to the diversity of methanotrophs (**a**; $r^2 = 0.59$, $P < 0.001$), and variability in the flux of CO_2 in relationship to total bacterial diversity (**b**; $r^2 = 0.02$, $P = 0.66$). Means and standard errors of the coefficients of variance for the annual flux are on the basis of measurements collected between 2006–2008. Detailed descriptions of symbols can be found in the legend to Figure 1: ▼ = AG, ■ = ES, ▲ = SF, ◆ = MG, ● = DF. Error bars represent standard errors.

richness (Chao, 1984) to compare diversity. To control for unequal sequencing depths, we estimated richness in each sample at 6600 sequences, the Chao 1 estimate from our lowest sequenced sample. There was no obvious relationship between CO_2 production and estimated bacterial diversity ($P = 0.522$ for linear regression, Figure 2b).

Influence of diversity on stability

To assess variability in the flux of CO_2 and CH_4 across treatments, we compared the coefficient of variance in the annual fluxes for each land management practice in each year between 2006 and 2008, the years for which molecular data was collected. The variance in CH_4 was negatively correlated with methanotroph diversity ($P < 0.001$): sites with the highest richness had the lowest variance (Figure 3a). Conversely, variation of CO_2 flux was not correlated with overall bacterial diversity (Figure 3b).

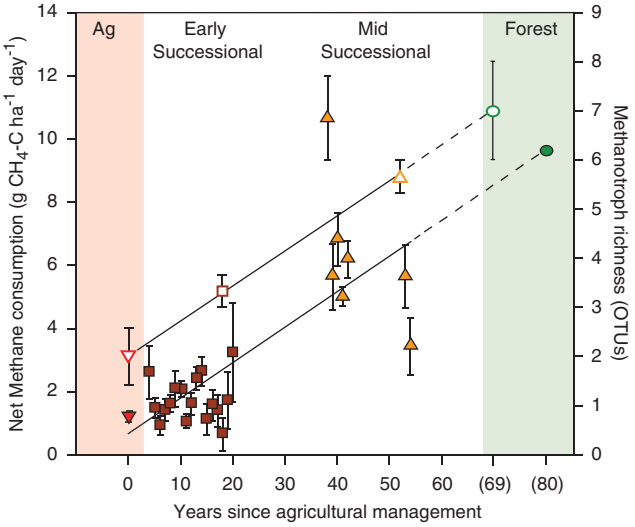

Figure 4 The recovery of methanotroph diversity and CH_4 consumption at KBS LTER following row-crop agriculture. Increase in methanotroph diversity (open symbols) and CH_4 consumption (closed symbols) as a function of time since cessation of agriculture. Measurements of the deciduous forest (DF) are positioned on the basis of projections from linear regression used to fit methanotroph diversity ($y = 0.07x + 2.05$; $r^2 = 0.99$, $P = 0.020$) or CH_4 consumption ($y = 0.11x + 0.67$; $r^2 = 0.56$, $P < 0.001$). All measurements (diversity and consumption) are annual averages with error bars representing standard errors. Detailed descriptions of symbols can be found in the legend to Figure 1: ▼ = AG, ■ = ES, ▲ = SF, ◆ = MG, ● = DF.

Recovery from agriculture

Given the relationship between methanotroph diversity and CH_4 flux, changes in the membership of the methanotroph community were assessed following the release of lands from row-crop agriculture. The community of methanotrophs differed following the abandonment of agricultural practices ($P < 0.005$, analysis of similarity), and soils began to recover in terms of CH_4 consumption and methanotroph diversity (Figure 4). The methanotroph species present in the mid-successional plots began to overlap with those in fields that have not been used for agriculture (MG and DF) (Supplementary Figure S4).

There was a linear trajectory for the recovery of both CH_4 consumption ($r^2 = 0.56$, $P < 0.001$) and methanotroph richness ($r^2 = 0.99$, $P = 0.020$) over time with the slopes of these lines nearly parallel. Extrapolation of the linear relationships suggests that approximately 80 years would be required for full restoration of CH_4 consumption and methanotroph diversity to the levels of the current deciduous forest (Figure 4).

Discussion

Species richness is an integral component of community ecology, with the richness of plant species linked to productivity (Tilman *et al.*, 2001) and greater temporal stability in plant production

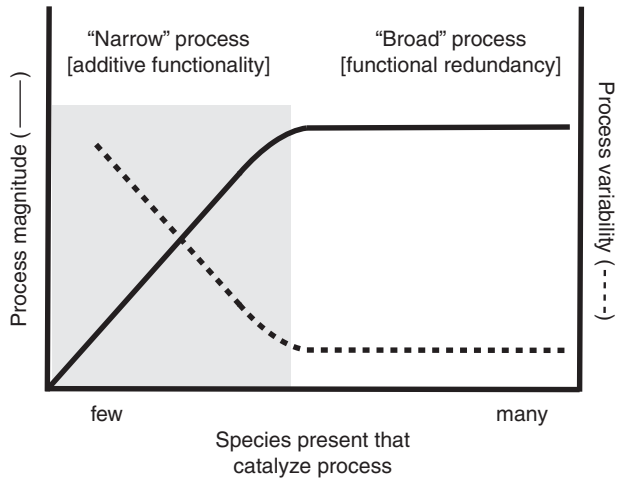

Figure 5 Conceptual model of the relationships between the number of species present in an environment that catalyze a particular ecosystem process (richness) and the magnitude and variability (1 per stability) of that process. Processes catalyzed by microbes with specialized metabolisms that are mediated by a few species are proposed to occur in the shaded area.

(Tilman *et al.*, 2006) across many landscapes (Wacker *et al.*, 2009). Although the mechanisms underlying these relationships for macroorganisms are vigorously debated and investigated, microbiologists are just beginning to ask whether functionality, stability and diversity are correlated in microbial communities (Konopka, 2009).

We investigated the relationship between microbial diversity and two ecosystem-level processes that are catalyzed by soil microbes—the consumption of CH_4 and the production of CO_2. On the basis of ecological models and considerations of biodiversity and microbial metabolism in soil (Schimel, 1995; Groffman and Bohlen, 1999; Zak *et al.*, 2003), we hypothesized that the relationship between microbial diversity and the magnitude and stability of gas fluxes would depend on the total number of species that are present to catalyze each process (Figure 5). If this model is true, the rate and stability of CH_4 consumption, which is a specialized metabolic process limited to relatively few species, would be positively correlated with methantroph richness, whereas CO_2 production, the result of general metabolic pathways shared by thousands of different heterotrophs in soil, would not be related to bacterial diversity.

As postulated, there was a positive relationship between rates of CH_4 consumption and the diversity of methanotrophs across a range of land uses at the KBS LTER (Figure 2a). The observed changes in methanotroph richness are consistent with other studies where methanotroph diversity increased in reforested farmland (Knief *et al.*, 2005; Dorr *et al.*, 2010) and reclaimed pasture lands (Zhou *et al.*, 2008), and where methanotroph richness was linked to CH_4 consumption in other sites (Lin *et al.*, 2005; Singh *et al.*, 2007; Degelmann *et al.*, 2010). Our study establishes a definitive correlation between methanotroph richness and CH_4 consumption across a successional gradient of landscapes from row-crop agriculture to deciduous forest.

Changes in methanotroph diversity and CH_4 consumption along this gradient highlight the strong and long-lasting impact of agricultural management. On the basis of recovery of CH_4 consumption following abandonment from agriculture, we project that it will require approximately 80 years for methanotroph diversity and CH_4 consumption to return to that of a native deciduous forest (Figure 4b). This slow recovery is consistent with worldwide observations that suggest a recovery period of 100 years for CH_4 consumption (Smith *et al.*, 2000).

In contrast, we found no observable relationship between bacterial diversity in soil and large differences in the production of CO_2 (Figure 2b). Given the extensive microbial diversity in soils and the ubiquitous distribution of CO_2-yielding metabolic pathways in heterotrophs, it is perhaps not surprising that there was no discernible relationship between diversity and soil respiration. The lack of a general relationship between bacterial diversity and soil respiration has been documented for other soil types as well (Balser and Firestone, 2005; Wertz *et al.*, 2006). Rather than being related to bacterial diversity, we found that CO_2 production from soil was more strongly predicted by soil moisture and temperature—two well-known regulators of soil metabolism (Cook and Orchard, 2008). Respiration of fungi, soil arthropods and plant roots also respond to these master regulators, making it difficult to disentangle the relative contribution from members of this complex community of organisms, but at the KBS LTER, bacterial diversity is not one of the driving factors.

Variability in both CH_4 consumption and CO_2 production are also consistent with the postulated model (Figure 5). Variation in CH_4 consumption was highest in agricultural plots that harbored the lowest diversity, whereas plots with the highest diversity were the most stable. We suggest that increased stability arises from the presence of complementary niches of methanotrophs. As environments vary temporally or spatially across a landscape, the presence of a more diverse assortment of methanotrophs that are able to oxidize CH_4 under a broader set of environmental conditions provides for more consistent CH_4 consumption. We also recognize that some variability may be contributed by technical limitations associated with the measurement of low rates of CH_4 consumption. Variability in CO_2 flux was smaller than for CH_4 flux, and there was no obvious relationship to bacterial diversity. As predicted by our model, once there is sufficient redundancy in a metabolic process, the gain or loss of species is not expected to influence the overall rate or stability of the process catalyzed by that metabolic group of microbes. It is noteworthy that both aspects of the model are captured in the same

sample: a broadly distributed process not linked to ecosystem function and a specialized, narrowly distributed process linked to the magnitude and stability of a process. This suggests that efforts to mediate the impact of agricultural practices on greenhouse gas flux is best focused on organisms and factors contributing to narrowly distributed processes.

Two hypotheses have been advanced to explain the underlying basis of the relationship between ecosystem-level processes and diversity: the magnitude of a process can be the result of the combined activities of species present (complementarity hypothesis), or the magnitude can be the result of the presence of one or a few particularly productive species more likely to be present when diversity is high (sampling hypothesis) (Loreau and Hector, 2001; Fargione *et al.*, 2007). Distinguishing between these competing hypotheses would determine whether all methanotrophs or only select species need to be conserved or restored in order to stimulate CH_4 consumption where it has been attenuated by change in land use. If cumulative diversity were of primary importance, as suggested in the complementarity hypothesis, strategies that stimulate the growth of all methanotrophs would be most effective. If, on the other hand, certain high-activity species are the primary drivers of CH_4 consumption, as suggested by the selection hypothesis, then strategies should be directed towards the establishment of those specific methanotrophs. The concurrent recovery of CH_4 oxidation and methanotroph diversity following the abandonment from row-crop agriculture (Figure 4) and the successive accumulation of methanotroph species in addition to those present in the agricultural site (Supplementary Figures S3, S4, Supplementary Table S1) is consistent with the complementarity of methanotrophs leading to increased functionality. Continued monitoring of the recovery of both methanotrophs and CH_4 consumption, coupled with functional assays to determine which methanotroph populations are most active throughout the year will provide a rigorous test of these alternative hypotheses.

The slow recovery of CH_4 consumption following the abandonment of agricultural management practices suggests that many dimensions of methanotroph niches are disrupted by row-crop agriculture. Recovery of the diversity of methanotroph communities has been observed in sites where plant diversity was increased (Knief *et al.*, 2005; King and Nanba, 2008; Zhou *et al.*, 2008; Degelmann *et al.*, 2010; Dorr *et al.*, 2010), perhaps due to the differential production of monoterpenes (Maurer *et al.*, 2008; Degelmann *et al.*, 2010), suggesting a role for plant diversity in shaping methanotroph communities. Additionally, nitrogen fertilization is known to change the structure of methanotroph communities (Lau *et al.*, 2007; Maxfield *et al.*, 2008), most likely through competitive inhibition of methane monooxygenase by ammonia (Gulledge and Schimel, 1998), although it can stimulate CH_4 consumption under conditions where fixed nitrogen is limiting methanotroph metabolism (Bodelier and Laanbroek, 2004). At KBS LTER, ammonia has been shown to cause acute short-term reductions in CH_4 consumption rates in DF and SF soils (Suwanwaree and Robertson, 2005). Within the land-use gradient at KBS LTER, differences in gas diffusivity (von Fischer *et al.*, 2009), the disruption of soil aggregates by tillage (Grandy and Robertson, 2007), nitrogen fertilization, as well as documented drivers of microbial diversity (including pH and salinity (Fierer and Jackson, 2006)), are additional factors that might be influencing methanotroph diversity and rates of CH_4 consumption. Identifying parameters that influence methanotroph diversity, including the influence of soil type and climate across sites, will be of vital importance if we are to manage lands to conserve or restore methanotroph diversity and enhance the capacity of soil to serve as a stable sink for atmospheric CH_4.

Conclusions

One of the most important drivers of species loss in terrestrial ecosystems worldwide is simplification of ecosystem structure that results from intensified land use (Sala *et al.*, 2000). We document a decrease in both methanotroph diversity and CH_4 consumption in soils managed for row-crop agriculture compared with native deciduous forests and never-tilled soils managed as grasslands. There was not a similar relationship between soil respiration and bacterial richness, consistent with the prediction that microbial diversity is more likely to be important in the case of specialized metabolic processes rather than broadly distributed types of metabolism and that specialized processes are better targets for microbial mediation. The concomitant recovery of methanotroph diversity and CH_4 consumption suggests that all methanotroph species contribute to CH_4 consumption as suggested by the complementarity hypothesis. Identifying parameters that impact methanotroph diversity will inform decisions about land management that have the potential to influence the flux of this potent greenhouse gas.

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