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Review paper

Long-term effects of mineral fertilizers on soil microorganisms – A review

Daniel Geisseler*, Kate M. Scow

Department of Land, Air and Water Resources, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA

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ABSTRACT

Increasing nutrient inputs into terrestrial ecosystems affect not only plant communities but also associated soil microbial communities. Studies carried out in predominantly unmanaged ecosystems have found that increasing nitrogen (N) inputs generally decrease soil microbial biomass; less is known about long-term impacts in managed systems such as agroecosystems. The objective of this paper was to analyze the responses of soil microorganisms to mineral fertilizer using data from long-term fertilization trials in cropping systems. A meta-analysis based on 107 datasets from 64 long-term trials from around the world revealed that mineral fertilizer application led to a 15.1% increase in the microbial biomass (C_{mic}) above levels in unfertilized control treatments. Mineral fertilization also increased soil organic carbon (C_{org}) content and our results suggest that C_{org} is a major factor contributing to the overall increase in C_{mic} with mineral fertilization. The magnitude of the effect of fertilization on C_{mic} was pH dependent. While fertilization tended to reduce C_{mic} in soils with a pH below 5 in the fertilized treatment, it had a significantly positive effect at higher soil pH values. Duration of the trial also affected the response of C_{mic} to fertilization, with increases in C_{mic} most pronounced in studies with a duration of at least 20 years. The input of N per se does not seem to negatively affect C_{mic} in cropping systems. The application of urea and ammonia fertilizers, however, can temporarily increase pH, osmotic potential and ammonia concentrations to levels inhibitory to microbial communities. Even though impacts of fertilizers are spatially limited, they may strongly affect soil microbial biomass and community composition in the short term. Long-term repeated mineral N applications may alter microbial community composition even when pH changes are small. How specific microbial groups respond to repeated applications of mineral fertilizers, however, varies considerably and seems to depend on environmental and crop management related factors.

1. Introduction

Mineral fertilizers, especially nitrogen (N) inputs, have been a major contributor to the impressive crop yield increases realized since the 1950s (Robertson and Vitousek, 2009). Nitrogen is also the limiting nutrient for primary production in many terrestrial ecosystems and increased N input often leads to higher net primary production (LeBauer and Treseder, 2008).

Below ground communities are also affected by inputs of nutrients to the soil. In a literature review, Allison and Martiny (2008) found that 84% of 38 studies reported that microbial community composition is sensitive to N, phosphorus (P), and potassium (K) fertilization. While net primary production in terrestrial ecosystems is generally N limited, soil microorganisms may be carbon (C) or N limited (Wardle, 1992). The response of soil microbes may therefore differ from the response of the plant community. In fact, recent meta-analyses based on data predominantly from unmanaged ecosystems suggest that increasing N inputs suppress soil microorganisms (Treseder, 2008; Liu and Greaver, 2010; Lu et al., 2011).

The sensitivity of soil microbial communities likely differs between unmanaged and agricultural ecosystems. In agricultural fields, fertilizer N inputs exceed rates of atmospheric deposition and fertilizer N is often added in one or just a few large applications per year. High application rates lead to temporarily very high osmotic potentials and potentially toxic concentrations of the N forms added (Eno et al., 1955; Omar and Ismail, 1999). At higher application rates, short- and long-term effects on soil pH may also be more pronounced in agricultural systems. In addition, with the exception of weeds, plant community composition in cropping

* Corresponding author. Tel.: +1 530 754 6497; fax: +1 530 754 1552.
E-mail address: djgeisseler@ucdavis.edu (D. Geisseler).

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systems is little changed by fertilization, and is also intensively managed, which is in contrast to the case in unmanaged ecosystems (Clark et al., 2007; Cleland and Harpole, 2010). Furthermore, the substantially higher productivity brought on by fertilization in agricultural systems increases inputs of organic material in the form of root exudates, decaying roots and aboveground residues, and thus, increases the pool of C sources for soil microorganisms. A conceptual model of these interactions in agricultural systems is presented in Fig. 1.

Long-term fertilization trials allow investigations of the effects of repeated additions of mineral fertilizer on soil microorganisms. Many long-term trials were established primarily to study the impact of fertilizers on crop production; however, an increasing number of scientists are taking advantage of these well documented experiments to study soil microbial communities under different fertilization regimes.

The objective of this review is to test the hypothesis that long-term fertilization of agricultural crops with mineral fertilizers leads to changes in soil microbial biomass and community composition. We synthesize the results from a number of long-term cropping systems trials to investigate both direct and indirect effects of mineral fertilizers on soil microbial communities. Our review focuses specifically on the effects of mineral N fertilizers. However, in most long-term trials, P and K are also applied. Therefore, the observed effects cannot be attributed solely to N inputs.

2. Material and methods

2.1. Selection criteria

To quantify the effect of long-term N fertilizer application on soil microorganisms, we analyzed results from peer-reviewed studies in a meta-analysis. We searched the online database Web of Science for papers using the keywords "long-term', 'fertil*', 'microbial', and 'nitrogen'. In addition, articles cited in review papers that analyzed data from long-term agricultural trials were included in our search.

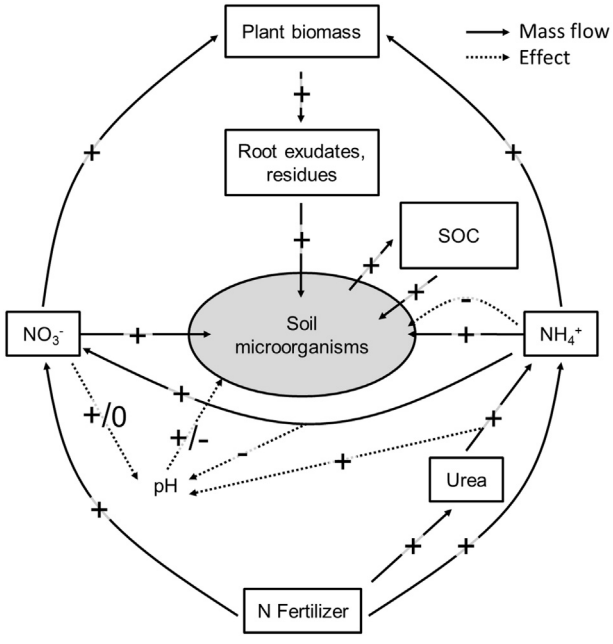

Fig. 1. Conceptual model of the direct and indirect effects of mineral N fertilizer on microorganisms in agricultural soils.

The following criteria were applied to select appropriate studies: (i) The data were from field trials with annual crops (except lowland rice cropping systems in paddy soils), (ii) the trials had been initiated at least five years prior to soil sampling, and (iii) the study reported microbial biomass and soil organic carbon (C_{org}) from an unfertilized control and a treatment with mineral N fertilization. Although urea is chemically an organic molecule, its behavior in soil is much more like that of a mineral fertilizer and we consider it as such in this review.

When studies reported data from several soil layers, only data from the topsoil were included. When different studies reported data from the same trial and treatments, the most recent dataset was included. Different crop rotations at one site were entered as individual datasets when an unfertilized control for that rotation was present. Treatments which differed in their N application rates and types of N fertilizers were also entered as individual treatments. In contrast, at sites where different tillage treatments were investigated in addition to N fertilization, only data from the conventional tillage plots were included. Some trials include treatments of different combinations of N, P or K fertilizers with identical N rates. In these cases, we only used data from the NPK treatment, which is the most common treatment in trials with only one mineral fertilizer treatment. Therefore, even though this review focuses on the effects of N fertilization, it is important to bear in mind that P and K fertilizers may have contributed to the observed effects.

A total of 107 datasets, each including an unfertilized control and a mineral N fertilizer treatment, from 64 long-term trials from across the world met our criteria and were included in the analysis (Table 1). Of these, 18 studies also reported specific respiration (qCO_2) values, and between 8 and 26 datasets included data on individual enzyme activities.

The sampling depth across all studies ranged from 5 to 50 cm, with 85% of the soil samples being taken to a depth of 15–20 cm. The duration of the trials ranged from 5 to 130 years, averaging 37 years. The annual N application rate ranged from 10 to 650 kg ha^{-1} averaging 136 kg ha^{-1}, with urea or ammonium salts being the most commonly used fertilizers. For more information about the trials and treatments, see Supplementary Tables S1 and S2.

Additional short- and long-term studies were included for the discussion to provide a broader perspective for the results of the meta-analysis and to investigate effects of fertilization on microbial community composition.

2.2. Data analysis

When organic matter was reported, we multiplied it by 0.59 to calculate C_{org}. Total PLFA was converted to microbial biomass C (C_{mic}) using a conversion factor of 5.8 mg C nmol^{-1} PLFA (Joergensen and Emmerling, 2006).

Table 1
Geographic location of the trials included.

Region	Trials	Datasets
Europe	10	16
North America	21	45
USA	*16*	*31*
Canada	*5*	*16*
Latin America	2	3
Australia	2	2
Asia	26	36
China	*13*	*14*
India	*12*	*21*
Africa	3	3
Total	64	107

The effects of N fertilizers on C_{org}, C_{mic}, qCO_2 and soil enzyme activities (protease, β-glucosidase, urease, acid and alkaline phosphatase) across studies were analyzed using meta-analysis. The natural log of the response ratio (RR) was used as effect size (Hedges et al., 1999):

$$\ln(RR) = \ln\left(\frac{X_{+N}}{X_{-N}}\right) \quad (1)$$

where X_{-N} and X_{+N} are the means of the target variable in the control and fertilized treatment, respectively. The analysis was performed on a spreadsheet using a random effect model according to equations presented by Rosenberg et al. (2013) and Rosenberg (2013). The correctness of the calculations was first tested using the dataset and step-by-step calculations described by Gurevitch and Hedges (2001).

Meta-analysis requires a variability estimate for each dataset. However, only about one in four studies reported standard deviations or another measure of variability that could be used to calculate the standard deviation. The missing standard deviations were calculated for each treatment and variable using the average coefficient of variation (CV) of the datasets where the standard deviation was reported. The average CV for C_{org}, C_{mic} and qCO_2 was 0.06, 0.13 and 0.11, respectively, while its average ranged from 0.05 to 0.10 for the different enzyme activities.

The standard deviation for the different ratios (e.g. C_{mic}/C_{org}) was calculated based on Ku (1966). When the two variables are correlated, an estimate of the covariance (COV) is required, which can be calculated as follows (Cohen et al., 2003):

$$COV = r^*\sqrt{s^2(X_{-N})^*s^2(X_{+N})} \quad (2)$$

where r is the correlation coefficient and $s^2(X_{-N})$ and $s^2(X_{+N})$ are the variances of the control and fertilized treatments, respectively. Soil organic C and C_{mic} have been found to be closely related among samples of the same field or among different fields with similar climate and cropping system, with r values generally exceeding 0.8 (Anderson and Domsch, 1989; Peigné et al., 2009). Strong correlations between enzyme activities and C_{org} have also been reported in a number of studies (Dick, 1984; Deng and Tabatabai, 1996; Klose and Tabatabai, 1999; Acosta-Martínez et al., 2003). To calculate the covariance, a correlation coefficient of 0.8 was used. A sensitivity analysis revealed that r had little effect on the confidence intervals. Entering r values ranging from 0.01 to 1 did not change the conclusions whether an effect was significant or not at the 5% level for any of the ratios analyzed.

3. Results

Across all datasets, the addition of mineral fertilization significantly increased C_{org} content compared to the unfertilized control by an average of 12.8% (Table 2). Only 17% of the datasets reported a lower C_{org} content in fertilized plots.

Fertilization significantly increased C_{mic} across all datasets by 15.1%. The effect of fertilization on C_{mic} was pH dependent (Fig. 2). While fertilization tended to reduce C_{mic} in soils with a pH below 5 in the fertilized treatment, it had a significant positive effect at higher soil pH values. In trials where soil pH in the fertilized treatment was at least 7, the fertilization-related increase in C_{mic} averaged 48% (Table 2). The duration of the trial also affected the response of C_{mic} to fertilization, with the increase in C_{mic} being highest in studies that had been in place at least 20 years. Across all datasets, the C_{mic}/C_{org} ratio was not significantly affected by fertilization (Table 2, Fig. 3).

Points below the regression line in Fig. 3 indicate that C_{mic} accounted for a smaller proportion of C_{org} in the fertilized treatment than in the control. This is especially pronounced in treatments of two trials where N was applied in the form of anhydrous ammonia application rates (Fig. 3; Table S2). One trial, located in western Oklahoma, received anhydrous ammonia application rates of 56, 168 and 504 kg ha^{-1} yr^{-1} (Deng et al., 2006). The trial at Scott, Saskatchewan, includes three N rates ranging from 45 to 180 kg ha^{-1} yr^{-1} and two fertilizers, namely anhydrous ammonia and urea (Biederbeck et al., 1996). At any N level, C_{mic} was considerably lower in the anhydrous ammonia than urea treatment. In both trials, C_{mic} decreased with increasing N additions. These two trials are the only ones in our dataset with anhydrous ammonia additions. While this is a small sample size, the data suggest that anhydrous ammonia may have a more negative effect on the microbial biomass than other N fertilizers.

The reasons for strong positive effects of fertilization on the C_{mic}/C_{org} ratio relative to the control are less clear. Trials where the C_{mic}/C_{org} ratio in the fertilized plots is at least 20% higher than in the control form a very diverse group (Tables S1 and S2). The types of N fertilizer used include urea, ammonium nitrate and ammonium sulfate, while the application rates ranged from 20 to 651 kg ha^{-1} yr^{-1}. Similarly, soil pH in the fertilized plots ranged from 4.5 to 8.1, and the latitude from 16° 30′ N to 53° 7′ N plus two trials in the southern hemisphere. The trials also represent considerable variation with respect to duration, crops grown, complexity of crop rotation and soil types.

In contrast to C_{mic}, qCO_2 tended to be lower in the fertilized soils. The activities of β-glucosidase and acid phosphatase were significantly higher in the fertilized plots (Fig. 4). Other enzyme activities

Table 2
Effects of mineral fertilizers on soil organic carbon (C_{org}), microbial biomass C (C_{mic}), and specific respiration (qCO_2). The microbial parameters in an unfertilized control (−N) were compared with a fertilized treatment (+N). For the meta-analysis, the response ratio (RR), its natural logarithm as well as the 95% confidence interval (CI) were calculated.

Property	Unweighted average			Meta-analysis			Significance level
	n	−N	+N	RR	ln RR	95% CI of ln RR	
Soil organic carbon (g kg^{-1})	107	14.5	15.7	1.128	0.120	0.095 to 0.146	***
Microbial biomass carbon (mg kg^{-1})							
All datasets	107	238	268	1.151	0.141	0.069 to 0.212	***
pH in +N treatment <5	17	240	213	0.969	−0.031	−0.41 to 0.347	n.s.
pH in +N treatment 5–7	39	234	253	1.093	0.089	0.012 to 0.166	*
pH in +N treatment 7 or higher	17	139	205	1.482	0.393	0.253 to 0.533	***
Duration 5–10 years	18	300	239	0.773	−0.258	−0.487 to −0.028	*
Duration 10–20 years	34	227	270	1.172	0.159	0.07 to 0.248	***
Duration 20 years or longer	55	224	276	1.305	0.266	0.201 to 0.332	***
C_{mic}/C_{org} (mg g^{-1})	107	21.5	22.6	1.021	0.020	−0.046 to 0.087	n.s.
Metabolic quotient (mg CO_2–C g^{-1} C_{mic} h^{-1})	18	7.6	6.6	0.909	−0.096	−0.194 to 0.002	n.s.

Significance level: * = $p < 0.05$, ** = $p < 0.01$, ** = $p < 0.001$, n.s. = not significant.

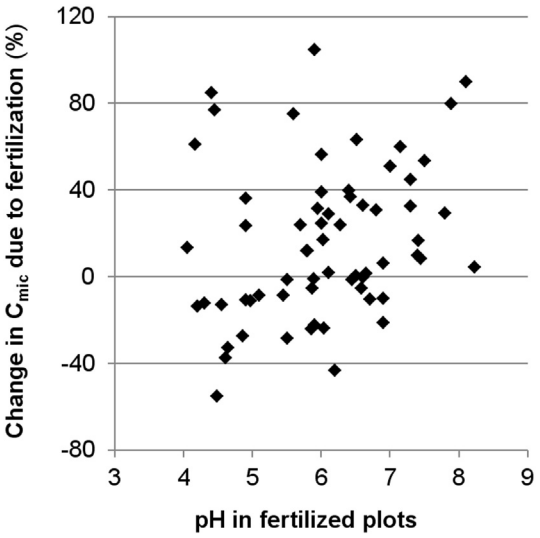

Fig. 2. Effect of fertilization on microbial biomass carbon (C_{mic}) as related to the soil pH in the fertilized plots.

tended to be higher in the fertilized plots, but differences compared to the unfertilized control were not significant. When corrected for differences in C_{org}, β-glucosidase activity was still significantly increased by fertilization, whereas alkaline phosphatase was reduced. Fertilization had no significant effect on the activities per unit C_{org} of protease, urease and acid phosphatase.

Our results suggest that the higher C_{org} content in the fertilized treatments is a major factor contributing to the overall increases in C_{mic} and enzyme activities. However, when fertilization reduces soil pH below a certain threshold, C_{mic} does not respond to fertilization or may even be reduced. Therefore, indirect effects of fertilization, such as increased C_{org} and lower pH seem to have a stronger effect on C_{mic} than do any direct effects of the fertilizer material applied. In contrast to C_{mic}, qCO_2 is reduced by mineral fertilization, which may be due to stress or a shift in the microbial community composition. These findings are discussed in detail in the following sections.

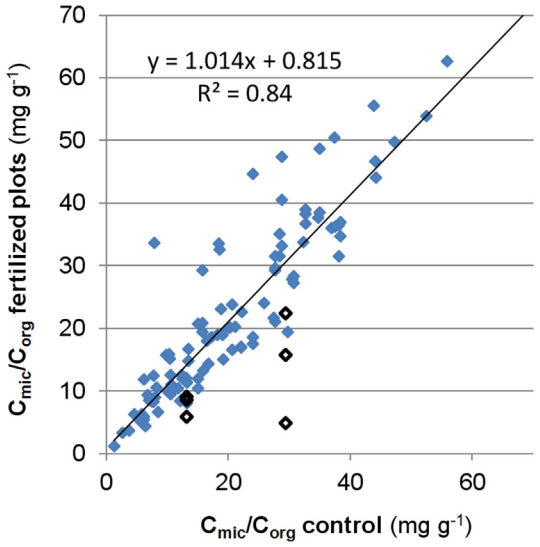

Fig. 3. Microbial biomass carbon (C_{mic}) to soil organic carbon (C_{org}) ratio in unfertilized soils plotted against the C_{mic}/C_{org} ratio in soils fertilized with mineral fertilizer. The white symbols with the black border are treatments with anhydrous ammonia additions. All data points were included in the regression analysis.

4. Discussion

4.1. Overall effect

Across all datasets included in the meta-analysis, C_{mic} was 15.1% higher in fertilized compared to unfertilized plots. This result agrees well with a meta-analysis of agricultural systems, where inorganic fertilizer use increased microbial biomass C per kg soil significantly, by 9%, above levels in unfertilized controls (Kallenbach and Grandy, 2011).

However, these results for agricultural systems contrast with studies carried out predominantly in unmanaged ecosystems. A synthesis of 82 field studies revealed that N fertilization reduced the microbial biomass by an average of 15% (Treseder, 2008). Likewise, Liu and Greaver (2010) found that N addition reduced C_{mic} by 20% across 57 studies. In another meta-analysis, Lu et al. (2011) reported that N additions significantly decreased microbial biomass N by 5.8%, with the effect being greatest in studies with a duration of 5–10 years. The decrease was much less pronounced in studies lasting longer than 10 years. In our meta-analysis we found a similar interaction with study duration, with the positive effect of fertilization on C_{mic} being greater in longer studies. However, this result contrasts with Treseder (2008) who found that longer durations of N fertilization elicited stronger declines in microbial abundance. Therefore, duration alone cannot explain the contrasting results measured in cropping systems and unmanaged ecosystems.

One factor that may explain the contrasting results is that, unlike in agricultural systems, N additions to unmanaged ecosystem often lead to changes in plant species composition and diversity, which in turn may affect the microbial community (Clark et al., 2007; Cleland and Harpole, 2010). Furthermore, N input can decrease soil pH, leading to the mobilization of aluminum and the leaching of nutrient cations (Vitousek et al., 1997). In the studies included in our analysis, soil pH only decreased by an average of 0.26 units (Table S2). This relatively small change is likely due to the fact that lime is often added in agricultural systems to buffer soil pH. Furthermore, depleted nutrient pools are replenished with fertilizers in agricultural systems. The uncorrected decline in pH in unmanaged systems associated with nutrient leaching and aluminum toxicity may explain why Treseder (2008) found that the decrease in the microbial population was more pronounced in longer trials.

The metabolic activity did not significantly respond to mineral fertilization. It is difficult to tease out how much any changes are specifically due to the fertilizers because a number of different factors may affect qCO_2. For example, physiological stress, physical disturbances, as well as a shift in the microbial community composition all may affect qCO_2. While a decrease in pH often results in an increased qCO_2 (Wardle and Ghani, 1995; Anderson, 2003), fertilization was found to either increase or decrease qCO_2 values depending on whether fertilization alleviates or increases stress (Wardle and Ghani, 1995). Furthermore, an increase in the fungal to bacterial biomass ratio has also been found to result in a lower qCO_2 value (Sakamoto and Oba, 1994; Blagodatskaya and Anderson, 1998). Our results suggest that either these factors were not strong enough across all studies to affect the C use efficiency of the microbial biomass, or that different effects canceled each other out. Unfortunately, the number of studies we found that measured qCO_2 is too small to isolate the contribution of different potential effects.

Despite the fact that the number of studies that reported enzyme activities was relatively small (Table 2), it is interesting to note that protease activity was not reduced in soils receiving mineral fertilizer despite evidence that protease production is

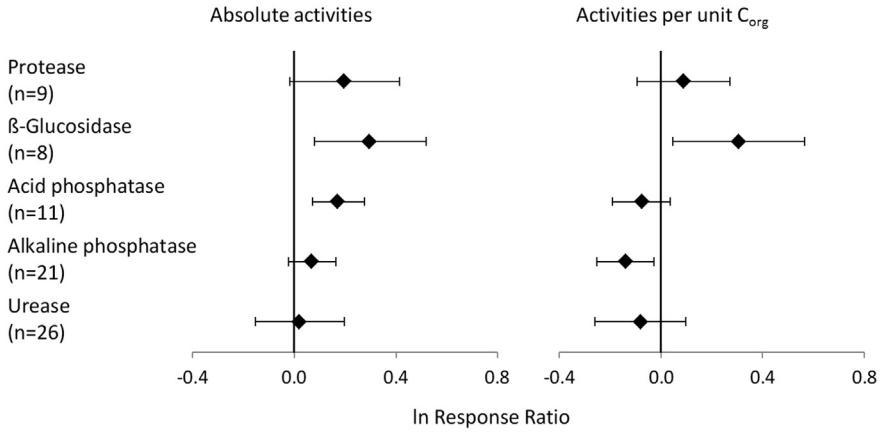

Fig. 4. Effect of fertilization on measured enzyme activities (left panel) and enzyme activities relative to the organic carbon content (C_{org}) of the corresponding treatments (right panel). Diamonds refer to the ln response ratio and the error bars represent the 95% confidence intervals. Confidence intervals overlapping with the vertical line drawn at zero indicate no significant effect of mineral fertilization.

generally repressed by ammonium (Glenn, 1976; Allison and Macfarlane, 1992). In contrast, a number of studies found an increased availability of mineral N promoted the production of extracellular enzymes involved in the C cycle (Henriksen and Breland, 1999; Carreiro et al., 2000; Geisseler and Horwath, 2009). This is in line with our results which showed a strong increase in β-glucosidase activity in fertilized soils (Fig. 4). In contrast, urease activity was little affected by fertilization, even though urea is a common N fertilizer.

4.2. Changed soil organic matter content

4.2.1. Mineral fertilizers and C_{org}

Long-term fertilization trials highlight the contribution of mineral fertilizers to the yield increases in crop production over the past decades (see for example Merbach and Körschens, 2002; Rothamsted Research, 2006). One notable exception is when ammonium or urea fertilizer applications result in pH values that drop below approximately 5. In such cases, yield can be significantly depressed and even drop below the unfertilized control (Zhang et al., 2008; Wessén et al., 2010; Schroder et al., 2011).

With increased productivity, the amount of plant residues returned to the soil after the crop is harvested also increases, which over the years has a positive effect on the soil organic matter content. Ladha et al. (2011) analyzed data from 104 long-term trials in agricultural systems throughout the world ranging in duration from 6 to 158 years. They found that the average C_{org} content in these studies decreased over time; however, the decrease was less pronounced in plots that received mineral N compared to the unfertilized control. Based on paired comparisons, C_{org} was on average 8% higher in plots receiving mineral N fertilizer compared to the control. Körschens et al. (2013) obtained a similar result analyzing data from agricultural long-term experiments located in Europe, which had been in place for 16–108 years. In their analysis, mineral NPK fertilization increased C_{org} by 10% compared to the control (Körschens et al., 2013). These results agree well with the studies included in our analysis where C_{org} was increased by an average of 8.5%.

4.2.2. Soil organic matter and soil microorganisms

In agricultural and unmanaged ecosystems, microbial biomass has been found to be strongly related to C_{org} concentrations (Booth et al., 2005; Cleveland and Liptzin, 2007; Fierer et al., 2009; Kallenbach and Grandy, 2011). This close relationship suggests that the fertilizer-induced increase in C_{mic} observed in our analysis was mainly due to the higher C_{org} in fields receiving mineral fertilizers. This hypothesis is supported by the fact that mineral fertilizer had no significant effect on the proportion of C_{org} contributed to by microbial biomass.

4.3. Soil pH

4.3.1. Mineral N fertilizers and soil pH

Though urea and ammonia fertilizers may temporarily increase soil pH over the long term (see next chapter), use of these fertilizers leads to decreases in soil pH, a fact that has been known for decades (Pierre, 1928). Acidification results from nitrification, the oxidation of ammonium to nitrite and then to nitrate, which produces protons. A survey of 10 long-term monitoring field sites in China revealed that soil pH decreased substantially by 0.45–2.20 units over an 8- to 25-year period in plots fertilized with mineral N, P and K fertilizers. In contrast, soil pH did not change in unfertilized control and fallow plots (Guo et al., 2010). In these trials, N was applied primarily in the form of urea and ammonium. Across all datasets in our analysis, the fertilizer-induced decrease in pH was moderate. This may be due to the fact that, in some cases, N was applied in the form of nitrate and, in other cases; soil pH was kept within a certain range with lime applications.

In several long-term trials, urea and ammonium fertilizers were found to reduce soil pH, while the application of nitrate had little effect on soil pH compared to unfertilized controls (Volk and Tidmore, 1946; Wolcott et al., 1965; Malhi et al., 2000). Striking differences in the effects of ammonium versus nitrate fertilizers on soil pH were observed in the long-term trial at Ultuna, Sweden. Between 1956, when the experiment began, and 1974, the pH in the topsoil of the ammonium sulfate treatment had dropped from an initial 6.5 to 5.0 (Kirchmann et al., 1994). Since then the pH has decreased even further, dropping to 4.2 in 2009. In contrast, in both the unfertilized and calcium nitrate plots, the pH hadn't changed much from the initial values, poised at 6.2 and 6.7 in 2009, respectively (Börjesson et al., 2012).

The acidifying effect of N fertilization also depends on N application rate. With increasing rates of application, the decrease in pH per unit of applied N is more pronounced. This is due to the fact that some of the acidity produced by nitrification is neutralized when plants take up more nitrate than cations (Barak et al., 1997). When excess N is applied, however, the proportion of N taken up, and hence the neutralizing effect of nitrate uptake, is reduced. Long-

term trials at Arlington, Wisconsin, and Manhattan, Kansas, have clearly shown a direct relationship between N application rate, applied as either urea or ammonium nitrate, and acidification (Schwab et al., 1990; Barak et al., 1997).

4.3.2. Effect of soil pH on soil microorganisms

Studies carried out in a number of ecosystems have shown that pH exerts a strong influence on the composition of soil microbial communities. In 98 soil samples collected in different ecosystems in North and South America, Fierer and Jackson (2006) found that the diversity and richness of soil bacterial communities (by pyrosequencing) differed substantially across ecosystem types and that differences in soil pH largely explained the variation. Differences in bacterial community composition were most pronounced in soils with a pH below 5. Bacterial diversity was highest in neutral soils and lower in acidic soils. In 53 soil samples collected from mature broad-leaf forests, with pH values ranging from 3.0 to 7.2, Bååth and Anderson (2003) found a positive correlation between soil pH and microbial biomass. Furthermore, soil pH had a strong effect on community composition (by PLFA) in these soils. In 88 soils collected across North and South America, with pH values ranging from 3.6 to 8.9, Lauber et al. (2009) similarly found that differences in bacterial community composition (by pyrosequencing) were significantly correlated with differences in soil pH.

4.3.3. Results from long-term trials

Several studies investigated the effect of ammonium and nitrate fertilizers on soil microorganisms at the long-term trial at Ultuna. While the microbial biomass was generally increased in the plots fertilized with calcium nitrate compared to the control, it was reduced in the soil fertilized with ammonium sulfate, reflecting the changes in soil pH (Witter et al., 1993; Marstorp et al., 2000). Repeated application of calcium nitrate had little effect on the bacterial (Elfstrand et al., 2007; Wessén et al., 2010; Harris et al., 2012) and fungal biomass (Marstorp et al., 2000; Enwall et al., 2005) and the amounts of individual PLFA as well as gene abundance did not differ significantly between the unfertilized control and the treatment fertilized with calcium nitrate (Hallin et al., 2009; Börjesson et al., 2012). In contrast, the abundance of bacteria and archaea was significantly reduced in the soil fertilized with ammonium sulfate (Hallin et al., 2009; Wessén et al., 2010), while the fungal biomass was much less affected (Marstorp et al., 2000; Börjesson et al., 2012). In addition, basal respiration and qCO_2 were found to be significantly increased in the ammonium sulfate treatment compared to the control and the nitrate treatment (Enwall et al., 2005, 2007). The comparison of these three treatments suggests that the input of N per se has little effect on the microbial biomass. The application of N in the form of ammonium, however, strongly affects the microbial community. This may be due to the low pH or toxic effects of the repeated applications of ammonium, the two of which unfortunately cannot be distinguished clearly in this trial.

The effects of soil pH on soil microbial communities has been investigated at the Hoosfield acid strip, at Rothamsted, U.K. where an uneven application of lime in the 19th century resulted in a pH gradient ranging from 3.7 to 8.3 along a 200 m transect. No other organic or chemical amendments have been applied since then (Rousk et al., 2009). Microbial biomass C increased with increasing pH; however, the most pronounced effects were observed in soils with a pH below 5. Respiration was also reduced in soils with pH less than 4.5 and the respiration quotient increased in soil with a pH of 4 and below (Aciego Pietri and Brookes, 2008). The authors attributed these effects to pH-related stress. The total mass of bacterial PLFAs decreased only slightly as pH decreased between 8.3 and 5, however, the PLFAs decreased sharply below a pH of 5. A similar response to pH was also evident for total PLFA and SIR biomass. The fungal PLFA biomarkers were highest at an intermediate pH of 5.5–6.5 (Rousk et al., 2009).

Ramirez et al. (2010) and Fierer et al. (2012) separated the importance of different factors potentially influencing soil biology by comparing shifts in bacterial communities in a 27 year old grassland, receiving ammonium nitrate ranging from 0 to 800 kg ha^{-1} yr^{-1} and an 8 year old agricultural field receiving ammonium nitrate ranging from 0 to 267 kg N ha^{-1} yr^{-1}. The grassland soil pH decreased with increasing N fertilization rates from 7.4 to 6.1, while it decreased from 6.6 to 5.3 in the agricultural field. At both sites, microbial biomass C tended to increase with increasing inputs of fertilizer. An analysis of the bacterial community composition suggested that observed differences resulted mainly from differences in C and N availability, and to a lesser degree from shifts in the plant community or soil pH brought on by fertilization (Ramirez et al., 2010; Fierer et al., 2012). Furthermore, increased N additions did not have consistent effects on the richness and diversity of the soil bacterial community (Ramirez et al., 2010).

The results summarized in this chapter suggest that the application of N per se does not lead to a pronounced negative effect on soil microorganisms in agricultural systems. When N fertilization decreases the soil pH, however, soil microbial biomass, their activity and community composition are indeed affected. In unmanaged as well as in cropping systems, soil pH seems to have a particularly strong effect on soil microorganisms below a threshold pH of around 5.

4.4. Direct effects of mineral fertilizers

4.4.1. Mechanisms

Ammonium is the preferred nitrogen source for most bacteria and fungi (Merrick and Edwards, 1995; Marzluf, 1997). However, when applied at high rates, urea and ammonium fertilizers can inhibit soil microorganisms due to toxicity of ammonia, increases in pH and increases in ionic strength (Eno et al., 1955; Omar and Ismail, 1999).

The application of urea and ammonium fertilizers can lead to very high local concentrations of ammoniacal N (=NH_3 and NH_4^+). Concentrations exceeding 3000 mg g^{-1} soil next to a granule of urea (Yadvinder-Singh and Beauchamp, 1988) and 500 ppm next to the line of anhydrous ammonia injection (Eno et al., 1955) have been reported. Such high concentrations can strongly inhibit or kill fungi and bacteria (Eno et al., 1955; Setua and Samaddar, 1980; Omar and Ismail, 1999). However, some microorganisms, such as cellulolytic fungi, as well as some bacteria, can tolerate high ammonia concentrations (Omar and Ismail, 1999; Müller et al., 2006). Anhydrous ammonia, aqua ammonia and urea also increase soil pH considerably when the ammonia is converted to ammonium. Yadvinder-Singh and Beauchamp (1988) reported soil pH values of as high as 9 in the immediate vicinity of urea granules. Furthermore, the ionic strength of the soil solution may be high close to fertilizer granules and bands (Müller et al., 2006).

The harsh conditions created by these fertilizers are generally spatially limited. Yadvinder-Singh and Beauchamp (1988, 1989) found that the zone of high pH and ammoniacal N concentration created by fertilization did not extend beyond 6 cm of a large granule of urea. Eno et al. (1955) observed a similar pattern when applying anhydrous ammonia. However, Hou et al. (2010) reported ammoniacal N concentrations of 300 μg g^{-1} soil in a cabbage field where urea was broadcast and incorporated. In addition to being spatially limited, the concentration of ammoniacal N decreases within a few days or weeks in aerated soil due to nitrification and plant uptake (Eno et al., 1955; Hou et al., 2010; Pelster et al., 2011).

Even though long-term studies have shown that ammonium concentration may be higher in fertilized than unfertilized soils (He et al., 2007; Shen et al., 2008; Stange and Neue, 2009; Ai et al., 2013), concentrations at most times and in most locations are likely far below levels toxic to microorganisms.

4.4.2. Short-term effects

Despite the development of localized conditions hostile to soil biology following application of urea and ammonium fertilizers, short-term effects of fertilizer applications on soil microbial communities as a whole have been found to be minimal. Application of approximately 100 kg N ha^{-1} as urea in a laboratory incubation caused changes in microbial community composition evident at 10 days; however, the effects were not sustained over the 91-day incubation period (Stark et al., 2007). Similarly, in a greenhouse experiment with different soil types and crops, fertilization with ammonium nitrate had no significant effect on bacterial community structure in the rhizosphere (Marschner et al., 2001).

Urea applications at rates up to 90 kg ha^{-1} to no-till barley had no consistent effect on microbial biomass and bacterial functional diversity in two field trials in Canada (Lupwayi et al., 2011, 2012). The application of 120 kg N ha^{-1}, however, tended to decrease microbial biomass and functional diversity (Lupwayi et al., 2011). Similarly, a 3-year California study in a cotton-cereal rotation found no differences in microbial PLFA profiles between treatments with urea-N additions of 20 or 130 kg ha^{-1} (Roberts et al., 2011).

Fewer studies have investigated effects of nitrate fertilizers on soil communities. Addition of 100 and 2000 μg N g^{-1} soil as potassium nitrate had no significant effect on microbial biomass compared to the unfertilized control in a laboratory incubation of soils collected under annual crops, (Yevdokimov et al., 2008, 2012). At the higher rate, nitrate decreased the specific growth rate of the microbial community, increased the ratio of fungal to bacterial PLFA markers and decreased the ratio of Gram-positive and Gram-negative bacterial PLFA markers (Yevdokimov et al., 2012). The higher rate is not agronomically realistic; assuming incorporation into the top 5 cm of a soil with a bulk density of 1.35 kg m^{-3}, application of 2000 μg N g^{-1} soil would correspond to 1350 kg N ha^{-1} which far exceeds typical rates of fertilization in any system.

To sum up, changes in the soil environment resulting from application of urea and ammonium fertilizers can strongly affect soil microbial biomass and community composition in the short term. In contrast, short-term effects of nitrate seem to be much less pronounced. Also results suggest that soil microorganisms either recover quickly from potentially harmful effects of urea and ammonium fertilizers or that susceptible species are rapidly replaced by more tolerant ones, leading to no major differences in biomass or activity at the community level in the long term. Whether repeated application of N fertilizers may lower the ability of the microbial community to recover after N fertilizer application and lead to permanent changes in the microbial community is discussed in the following section.

4.4.3. Long-term effects

With strong indirect effects of fertilizers on soil microbial communities due to increased C_{org} content and lower pH, it is difficult to isolate long-term direct effects on soil microorganisms of repeated applications of N fertilizer. However, in two trials where N was applied as anhydrous ammonia, the microbial biomass was strongly decreased (Fig. 3; Biederbeck et al., 1996; Deng et al., 2006), suggesting that anhydrous ammonia may directly affect soil microorganisms. Our dataset also includes a number of research trials that included soils with pH values between 6 and 8.5 and in which the pH did not change more than 0.5 units between the control and the fertilized soil. In these cases, the indirect pH-related effects should be less pronounced and it should be possible to explore the direct effects of fertilizers. The following discussion focuses on these trials.

Several long-term studies found that fertilization led to changes in soil microbial community composition (Peacock et al., 2001; Böhme et al., 2005; Hartmann et al., 2006; Langer and Klimanek, 2006; Zhong et al., 2010; Hu et al., 2011; Kirchmann et al., 2013). Principal component analysis was generally used to determine the effects on the microbial community. However, a few studies found no or only small effects of mineral fertilization on soil microbial community composition (Esperschütz et al., 2007; Ogilvie et al., 2008; Börjesson et al., 2012). These results are in line with Allison and Martiny (2008), who found that 84% of studies with an average length of 8.2 years reported that microbial community composition was sensitive to N, P, and K fertilization.

The response of specific microbial groups to the long-term application of mineral fertilizers varies considerably. In general, fungi have been found to benefit from mineral N fertilization even when soil pH was little affected (Böhme et al., 2005; Esperschütz et al., 2007; Zhong et al., 2010; Ai et al., 2012; Zhang et al., 2012). However, Kirchmann et al. (2013) found a lower fungal biomass in the fertilized soil in two long-term trials in Sweden. They attributed this result to residues with a lower C to N ratio richer in N, which may have favored bacteria over fungi.

At the Martin Agricultural Experiment Station in Tennessee, the long-term application of ammonium nitrate resulted in a lower relative proportion of Gram-negative bacteria compared to in the unfertilized control, while the relative abundance of Gram-positive bacteria tended to increase (Peacock et al., 2001). The proportion of terminally branched saturated PLFA, which are used as biomarkers for Gram-positive bacteria, also increased in fertilized plots at both Bad Lauchstädt (Böhme et al., 2005) and Halle (Langer and Klimanek, 2006). However, the response of the bacterial community to mineral N fertilizer varied considerably among studies and no clear trends emerged. For example, at Ultuna and at the Broadbalk winter wheat trial, the bacterial community composition did not differ significantly between unfertilized soils and soils treated with mineral N fertilizer (Ogilvie et al., 2008; Hallin et al., 2009; Börjesson et al., 2012).

How the microbial community responds to mineral fertilizer is influenced by environmental and management related factors. Schneider et al. (2010) found that the most pronounced differences in fungal communities at the DOK trial were between samples collected in different years at different points in the crop rotation. In contrast, the influence of crops and farming systems, including fertilization, was smaller. Even when samples are taken at different times during one cropping season, sampling date can have a more pronounced effect on the soil microbial PLFA profile than long-term fertilization (Bossio et al., 1998). Analyzing the PLFA profile in soil samples from the long-term trials at Bad Lauchstädt and Keszthely, Böhme et al. (2005) found some similarities in the effect of fertilizers on the microbial community composition between the two sites, but also appreciable differences. The authors attributed these results to different site characteristics, including climate, soil properties and plant cover (Böhme et al., 2005). A comparison of four trials, including Halle and Bad Lauchstädt came to a similar conclusion (Langer and Klimanek, 2006). In fact, at Bad Lauchstädt, the prokaryotic diversity attached to large soil particles responded more strongly to fertilization than the community attached to clay minerals (Neumann et al., 2013). The authors concluded that clay fractions have a high buffering capacity which protects microbial cells against changes. The response of different microbial groups to fertilization may also differ between the bulk soil and rhizosphere soil (Ai et al., 2012). While fungi and Gram-negative bacteria

benefited most from mineral fertilizer applications in the bulk soil, the opposite was true, although to a much lesser and not significant degree, for the rhizosphere soil (Ai et al., 2012).

These results suggest that even in the absence of large pH changes, mineral fertilizer can have a significant effect on the microbial community composition. However, the effects may not be fully captured when focusing on the major groups, such as bacteria, fungi and actinomycetes. Furthermore, the response of specific microorganisms to mineral N additions may vary depending on environmental and management related factors.

4.4.4. Urea and ammonium fertilizers and nitrifying organisms

Ammonium oxidizing bacteria and archaea are catabolic specialists that require reduced forms of N, such as ammonium, as an electron donor. Therefore, the application of urea and ammonium should benefit ammonium oxidizers and nitrifying organisms. However, at very high concentrations, ammonia inhibits ammonium oxidizing bacteria and archaea (Prosser and Nicol, 2012).

In fact, in a laboratory study Koper et al. (2010) found that low ammonium concentrations of up to 1–2 mM increased nitrification rates. However, higher ammonium concentrations reduced the nitrification rate to almost half of the maximum (Koper et al., 2010). Yadvinder-Singh and Beauchamp (1988) also found that conditions within 2–3 cm of urea placement remained hostile for nitrifiers for at least 35 days, while nitrification occurred in the zone where the ammoniacal N concentration was below 1000 μg N g^{-1} soil.

Long-term studies suggest that the positive effects are more pronounced. Taking weekly field measurements at Bad Lauchstädt over the course of one year, Stange and Neue (2009) found that the average gross nitrification rate was higher in the treatment with mineral fertilization compared to the control. Several studies carried out at long-term trials in China also found that the potential nitrification activity was increased in soils with mineral fertilization compared to the unfertilized control (Chu et al., 2007; Shen et al., 2008; Ai et al., 2013).

These studies also revealed that ammonia oxidizing bacteria and archaea respond differently to mineral N fertilization. While the community of ammonia oxidizing archaea was little affected by mineral fertilization, the abundance and diversity of ammonia oxidizing bacteria was significantly increased (Chu et al., 2007; Shen et al., 2008; Ai et al., 2013). These three Chinese long-term trials all had soil pH values of 8 or higher and the repeated application of urea did not change the soil pH (at most reducing it by 0.5 units) below that of the unfertilized control. Therefore, these results may not be representative for all soils.

At Ultuna, in the ammonium sulfate fertilized treatment, Börjesson et al. (2012) found very low concentrations of PLFAs typically found in ammonia oxidizing bacteria and attributed this to the very low soil pH of 4.2 found in this soil (Börjesson et al., 2012). Soil pH changes also cause shifts in community composition; soils with a pH below 5.5 are generally dominated by ammonium oxidizing archaea (Prosser and Nicol, 2012). However, Hallin et al. (2009) found a reduced density of ammonia oxidizing bacteria and archaea per unit bacterial biomass at the same site. In another study carried out at Ultuna, Enwall et al. (2007) found that long-term fertilization with calcium nitrate significantly increased potential ammonia oxidation, while it was significantly decreased in the ammonium sulfate amended soils compared to the control.

He et al. (2007) also reported significantly reduced potential nitrification rates in a soil fertilized with mineral NPK fertilizers compared to the unfertilized control. As at Ultuna, the soil pH in the fertilized plot was significantly lower than in the control (4.04 vs. 5.78).

In a long-term trial initiated in 1961 at Craibstone, Scotland, in fields under a 8-year rotation, a series of plots were maintained at specific soil pH values ranging from 4.5 to 7.5 by addition of either lime or aluminum sulfate (Nicol et al., 2008). Archaeal *amoA* gene and transcript abundance decreased with increasing soil pH from 4.9 to 7.5, while bacterial *amoA* gene abundance showed the opposite trend. Across the entire pH gradient, archaeal genes and transcripts were more abundant than bacterial genes and transcripts (Nicol et al., 2008).

These studies suggest that in long-term trials, effects of N fertilization tend to have more positive than negative effects on the abundance and activity of ammonium oxidizers and other nitrifying organisms. The indirect effects of fertilization, especially changes in pH, however appear to have a greater effect on ammonium oxidizers and nitrifying organisms than do direct effects. Bacteria seem to benefit more than do archaea from N fertilization in agricultural soils, as long as soil pH does not decrease below approximately 5.5.

5. Conclusions

Our meta-analysis revealed that over the long term, fertilization of agricultural soil results in increased C_{mic} content, which is likely caused by associated increases in C_{org} due to higher crop productivity. The increased C_{mic} content observed in fertilized soils contrasts with what has been observed in unmanaged ecosystems, where C_{mic} often decreases as a result of N input. In trials of less than 10 years duration, C_{mic} was decreased by fertilization as well.

Urea and ammonium inputs can considerably lower soil pH over time, while nitrate fertilizers generally do not decrease pH. When the soil pH drops below approximately 5, there are negative effects not only on soil microorganisms but also on crop yields. A similar pH threshold for negative effects has been observed in unmanaged ecosystems.

In contrast to what has been reported in many unmanaged ecosystems, the input of N per se does not seem to have direct negative effects on soil microbial biomass in agricultural cropping systems. This may be due to the fact that N input does not change plant community composition in the way it often does in unmanaged ecosystems. However, long-term repeated mineral N applications can alter microbial community composition in many cases even when pH changes were small. These shifts in microbial community composition cannot be fully captured with methods that discriminate, at best, major microbial groups, such as bacteria, fungi, and actinomycetes. Genomics approaches that can differentiate changes within specific groups need to be used to identify and interpret phylogenetic and functional changes of microbial communities with long-term fertilizer induced changes.

How specific microbial groups respond to repeated applications of mineral fertilizers varies and seems to depend on environmental and crop management related factors. Based on the data available it is difficult to understand the interactions among environmental factors, fertilizer rates and types, and specific groups of soil microorganisms. More studies investigating the long-term effects of different fertilizers across different soil types and environmental conditions are needed to better understand these complex interactions. This is where the value of long-term experiments is evident in their ability to detect slow changes, such as those that were mentioned above. These changes might be missed in the 2- to 3-year duration of most studies.

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Appendix A. Supplementary data

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