Biol Fertil Soils (2010) 46:653–658
DOI 10.1007/s00374-010-0463-y

SHORT COMMUNICATION

Soil microbial properties under N and P additions in a semi-arid, sandy grassland

Lu-Jun Li · De-Hui Zeng · Zhan-Yuan Yu · Zhi-Ping Fan · Rong Mao

Received: 2 January 2010 / Revised: 13 April 2010 / Accepted: 19 April 2010 / Published online: 7 May 2010

Abstract Surface (0–15 cm) soil samples were collected from a semi-arid, sandy grassland in Keerqin Sandy Lands, Northeast China to study changes in soil microbial and chemical properties after five consecutive years of nitrogen (N) and phosphorus (P) additions. Nitrogen and P additions and their interactions negligibly affected soil organic carbon and total N contents, while P addition significantly increased soil total P content. Soil pH was significantly decreased by N addition, which significantly increased net nitrification rate, whereas it did not affect net N mineralization rate. No significant effects of N and P additions and their interactions on basal respiration were detected. In addition, N addition significantly decreased microbial biomass C (MBC) and N, and thus microbial quotient, but increased dissolved organic C and microbial metabolic quotient due to the significant decrease of MBC. Our results suggest that in the mid-term the addition of N, but not P, can change soil microbial properties, with a possible decline in soil quality of semi-arid, sandy grasslands.

Keywords Basal respiration · Microbial metabolic quotient · Microbial quotient · Net nitrogen mineralization · Nitrification · Soil microbial biomass

L.-J. Li · D.-H. Zeng (✉) · Z.-Y. Yu · Z.-P. Fan · R. Mao
Daqinggou Ecological Station, Institute of Applied Ecology,
Chinese Academy of Sciences,
72 Wenhua Rd,
Shenyang 110016, China
e-mail: zengdh@iae.ac.cn

L.-J. Li · R. Mao
Graduate University of Chinese Academy of Sciences,
Beijing 100049, China

Introduction

Human activities have altered global nutrient cycles and caused nutrient enrichment, especially nitrogen (N) and phosphorus (P), in many ecosystems (Mahowald et al. 2008; Vitousek et al. 1997). Nutrient enrichment can influence composition of biota and processes of the ecosystem. Matson et al. (2002) reviewed the consequences of N deposition for terrestrial ecosystems, and they suggested that not all ecosystems respond to N deposition similarly. In addition, P fertilization can lead to eutrophication of water by P runoff and leaching (Schoumans and Groenendijk 2000), but few studies have examined the ecological effects of P addition on terrestrial ecosystems (Bennett and Adams 2001; Stöcklin et al. 1998). Moreover, the effects of simultaneous N and P additions on terrestrial ecosystems remain uncertain (Elser et al. 2007) because of the limited number of studies. Soil quality is an important indicator in ecosystem management and sustainability (Masto et al. 2008), and thus, changes in soil quality due to nutrient additions need to be evaluated (Malý et al. 2009), so as to develop effective strategies for the management and sustainability of ecosystems under nutrient additions.

Soil microorganisms play a key role in sustaining soil quality, and soil microbial properties have been proposed as sensitive indicators of changes in soil quality (Filip 2002; Jenkinson 1988; Nannipieri et al. 2003). Soil microbial biomass serves as a source and sink of plant-available nutrients and is closely related to soil quality (Kaschuk et al. 2010). The microbial quotient (*q*MIC), the percentage of microbial biomass C (MBC) to soil organic C (SOC), can provide an effective indication of the improve-

ment or deterioration of soil quality (Wardle 1992). The microbial metabolic quotient (qCO_2), which is the amount of CO_2-C produced per unit MBC, has been used as an ecophysiological measure of ecosystem succession or disturbance (Wardle and Ghani 1995). Soil microbial biomass, *q*MIC, and qCO_2 have been used as indicators of soil development or degradation and changes in soil quality (Insam et al. 1989; Kaschuk et al. 2010). Potential N mineralization and nitrification have been frequently used to evaluate the N supply by soil (Robertson et al. 1999) and as indicators of ecosystem susceptibility to degradation (Aber et al. 1989).

Grasslands that are characterized by sandy soils constitute a large percentage of natural vegetation in arid and semi-arid regions of northern China. Located at the ecotone of agro-pastoral systems, the Keerqin Sandy Lands are one of the biggest sandy lands in China and are characterized by nutrient-poor soils. Rapid economic development in China has increased nutrient addition to soil throughout the country (Li et al. 2004; Lü and Tian 2007). To date, however, few data on the effects of nutrient addition on soil quality are available for this sandy grassland (Zeng et al. 2010). In this study, we have evaluated changes in soil microbial and chemical properties after 5 years of N and/or P additions in a sandy grassland in Keerqin Sandy Lands.

Materials and methods

Site and treatments

The experimental site was located at Daqinggou Ecological Station (42°58′ N, 122°21′ E, 260 m above sea level) of the Institute of Applied Ecology, Chinese Academy of Sciences, in the southeastern Keerqin Sandy Lands, Northeast China. The mean annual temperature was 6.4°C, the lowest mean monthly temperature (January) was −12.5°C, and the highest mean monthly temperature (July) was 23.8°C. The mean annual precipitation was 450 mm, with more than 60% occurring in June–August and a high potential evaporation of 1,300–1,800 mm per year. The plant community at the experimental site was dominated by *Pennisetum flaccidum*, *Chenopodium acuminatum*, *Cleistogenes chinensis*, *Artemisia scoparia*, and *Lespedeza davurica*. The soil type is Typic Ustipsamment, characterized by coarse texture and loose structure, with a texture of 90.9% sand, 5.0% silt, and 4.1% clay. The surface (0–15 cm) soil was slightly acidic (pH= 6.6) and the soil bulk density was ~1.5 g cm^{-3}.

The experimental site was a flat sandy grassland and was fenced to exclude livestock grazing. A factorial N × P addition experiment in a randomized block design with six replicates was established in 2004 and was based on four treatments: no addition (control), N addition (N), P addition (P), and combined addition of N and P (N + P). Nitrogen was added at the rate of 20 g N m^{-2} $year^{-1}$ as urea in 2004–2006 and as NH_4NO_3 in 2007–2008. Phosphorus was added at the rate of 4.4 g P m^{-2} $year^{-1}$ in the form of NaH_2PO_4 in 2004–2008. Nitrogen + P were added with the same forms and rates as N and P treatments. The fertilizer rate was similar to that normally used by farmers of the region. Each plot (4×4 m) was separated by a 2-m buffer strip. The fertilizers were dissolved in 16 L water and applied in early May (30%) and mid-June (70%) each year. Control plots only received 16 L of water without fertilizers.

Soil sampling

Soil samples were collected in April and July of 2009. After removal of the litter layer, eight soil cores (2.5 cm in diameter) were randomly collected from 0 to 15 cm layer in each plot and were mixed to form a composite sample. After removing visible roots, fauna, and organic debris by hand, the soil samples were sieved (<2 mm) and divided into two subsamples. One subsample was air-dried at room temperature (around 20°C) and used for chemical analyses. A portion of these samples was ground with a mill and sieved (<0.25 mm) prior to SOC, total N (TN), and total P (TP) analysis, whereas the other part was used for soil pH measurement. The other subsample was stored in plastic bags at 4°C for 24 h prior to analysis of NH_4^+–N and NO_3^-–N concentrations, net N mineralization and nitrification rates, basal respiration (BR), MBC, and microbial biomass N (MBN). The samples collected in July 2009 were only analyzed for SOC concentration.

Soil chemical properties

SOC concentration was determined by the $K_2Cr_2O_7$–H_2SO_4 wet oxidation method of Walkley and Black after removal of carbonates by acid pretreatment (Nelson and Sommers 1996). Total N and TP concentrations were determined with a continuous flow autoanalyzer (AutoAnalyzer III, Bran + Luebbe GmbH, Germany) after the samples were digested (340°C) with H_2SO_4 using a mixture of K_2SO_4 and $CuSO_4$ as catalyst. Soil pH on a 1:2.5 (*w*/*v*) mixture of soil and water was measured using a PHS-3C pH meter (Shanghai Lida Instrument Factory, China).

Soil N mineralization potential was measured by a laboratory incubation method described by Menyailo et al. (2003). Briefly, fresh soil (equivalent to 20 g oven-dry soil) was extracted with 2 M KCl solution (1:5 soil/solution ratio) to determine NH_4^+–N and NO_3^-–N concentrations with the continuous flow autoanalyzer. Additionally, another fresh soil (20 g oven-dry soil) was placed in a plastic flask, moistened to 60% of water-holding capacity, and aerobically incubated at 25°C for 15 days. To avoid

anaerobic conditions, the flask was opened every 3 days for 5 min. At the end of the incubation, the soil sample was analyzed for the final inorganic N concentration as described above. Soil net N mineralization rate (per day) was determined by the difference in inorganic N concentrations before and after the incubation. Likewise, net nitrification rate was calculated as the difference in NO_3^-–N before and after the incubation.

Soil microbial properties

Soil MBC and MBN were determined by the chloroform fumigation–extraction method (Cabrera and Beare 1993; Vance et al. 1987). Briefly, soil (25 g) was pre-incubated in a humidified, darkened, 25°C incubator for 7 days. Soils with or without chloroform fumigation were extracted with 50 mL 0.5 M K_2SO_4 for 30 min and then filtered. The organic C in the soil extract was measured by dichromate oxidation (Vance et al. 1987) and TN in the extract by $K_2S_2O_8$ oxidation method (Cabrera and Beare 1993). Microbial biomass was calculated as the differences in K_2SO_4-extractable C or N concentration between fumigated and unfumigated soils, divided by efficiency factors for MBC (K_C=0.38; Vance et al. 1987) and MBN (K_N=0.45; Jenkinson 1988), respectively. The unfumigated K_2SO_4-extractable organic C value was also considered as the soil dissolved organic C (DOC) concentration (Zeglin et al. 2007). The *q*MIC was calculated as a percentage of MBC to total organic C.

Basal respiration was determined by measuring CO_2 evolution (Hu et al. 2006). Briefly, field-moist soil (20 g oven-dry soil) was placed in 500-mL air-tight glass vessels and water was added to reach 60% of the water-holding capacity. The vessels were closed with rubber stopper and pre-incubated at 25°C for 3 days. Then, they were opened and closed again for a 24-h incubation period at the same temperature. The CO_2 evolved from the soil was absorbed in 10 mL 0.2 M NaOH, and the remaining base was titrated with 0.1 M HCl after the formed Na_2CO_3 was precipitated by adding 2 mL 1 M $BaCl_2$. The results were expressed as microgram CO_2-C per gram per hour. The qCO_2 was calculated by dividing the hourly BR by the corresponding MBC.

Statistical analysis

Changes in soil chemical properties (SOC, TN, TP, and pH) were analyzed by the two-way ANOVA using the General Linear Models procedure to test the main effects of N addition, P addition, and their interactions. Repeated measures ANOVAs were used to examine N addition and P addition effects on NH_4^+–N and NO_3^-–N concentrations, net N mineralization and nitrification rates, and MBC, MBN, BR, DOC, *q*MIC, and qCO_2 values at the two samplings. Between-subject effects were evaluated as N addition or P addition treatment and within-subject effects were time-of-season. All analyses were performed by the SPSS (version 13.0) and the accepted significance level was $\alpha = 0.05$.

Results

Nitrogen addition, P addition, and their interactions did not significantly affect SOC and TN concentrations (Table 1). The P addition significantly (P=0.002) affected TP concentration, whereas N addition and its interactive effects with P addition were not significant on TP concentration (Table 1). Nitrogen addition markedly (P<0.001) affected soil pH, while no significant interactive effect of N and P additions on soil pH was detected.

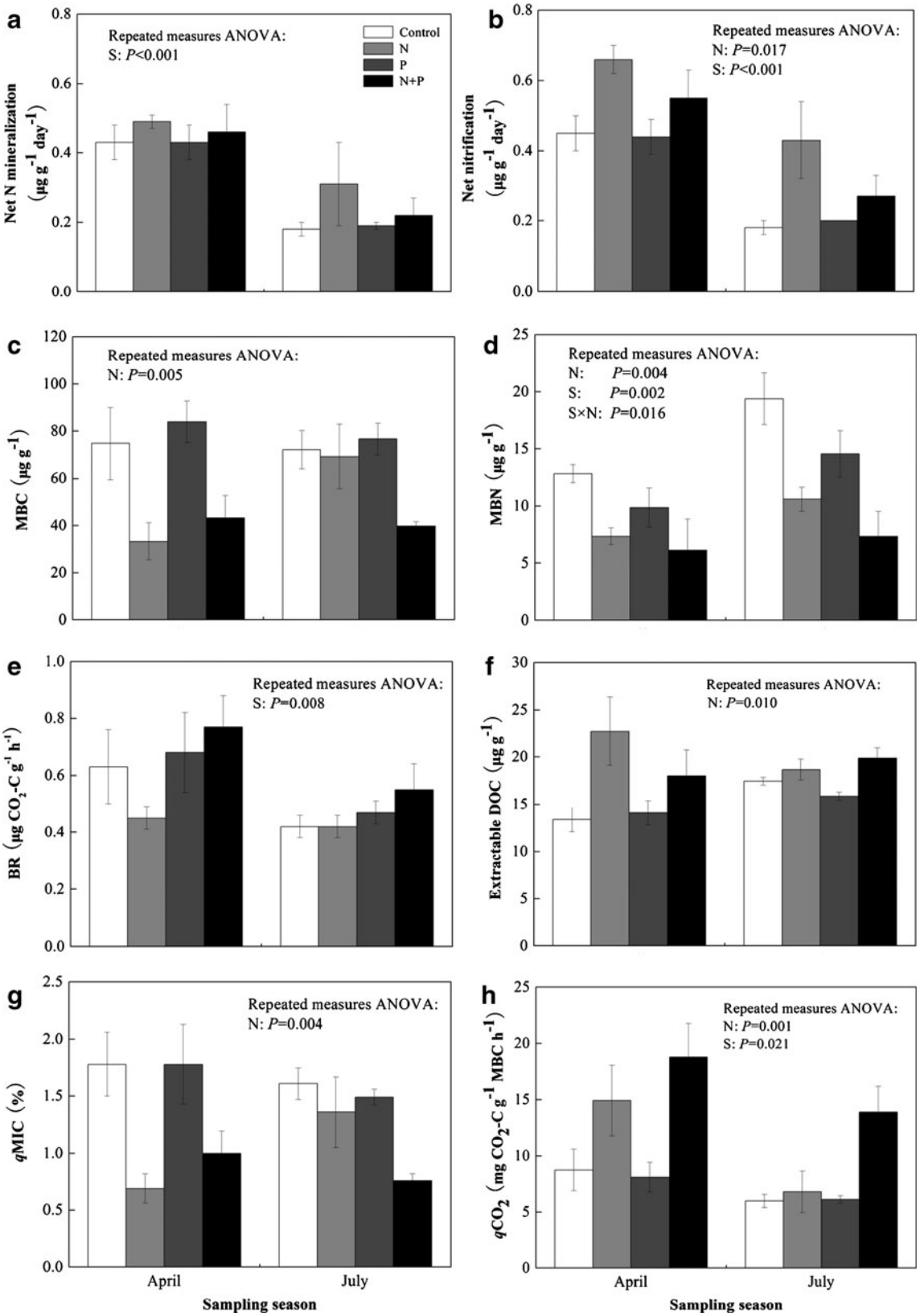
The effects of N addition, P addition, and their interactions were not statistically significant for net N mineralization rate, whereas N addition significantly enhanced net nitrification rate (P=0.017; Fig. 1a, b). No interactions were observed between sampling season and N and/or P addition on net N mineralization and nitrification rates (Fig. 1a, b).

The ANOVA analysis showed that N addition markedly decreased MBC (P=0.005; Fig. 1c), and thus, *q*MIC (P=0.004; Fig. 1g), but MBC and *q*MIC were not significantly affected by P addition (P=0.858 and P=0.587, respective-

Table 1 Concentrations of organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) and pH of surface (0–15 cm) soil under four treatments: control, nitrogen (N), phosphorus (P), and both nitrogen and phosphorus (N + P) additions

Treatment	SOC (mg g^{-1})	TN (mg g^{-1})	TP (mg g^{-1})	pH (H_2O)
Control	4.16±0.22	0.34±0.03	0.08±0.01	6.59±0.06
N	4.71±0.29	0.36±0.01	0.08±0.00	5.36±0.03
P	4.91±0.60	0.34±0.03	0.11±0.01	6.70±0.09
N + P	4.31±0.40	0.30±0.03	0.10±0.01	5.53±0.06
Two-way ANOVA results (*P* values)				
N	0.952	0.818	0.081	<0.001
P	0.671	0.369	0.002	0.051
N × P	0.191	0.369	0.219	0.644

Values are means ± SE, n=6


a
Repeated measures ANOVA:
S: P<0.001
Control
N
P
N+P
Net N mineralization (μg g-1 day-1)
b
Repeated measures ANOVA:
N: P=0.017
S: P<0.001
Net nitrification (μg g-1 day-1)
c
Repeated measures ANOVA:
N: P=0.005
MBC (μg g-1)
d
Repeated measures ANOVA:
N: P=0.004
S: P=0.002
S×N: P=0.016
MBN (μg g-1)
e
Repeated measures ANOVA:
S: P=0.008
BR (μg CO2-C g-1 h-1)
f
Repeated measures ANOVA:
N: P=0.010
Extractable DOC (μg g-1)
g
Repeated measures ANOVA:
N: P=0.004
qMIC (%)
h
Repeated measures ANOVA:
N: P=0.001
S: P=0.021
qCO2 (mg CO2-C g-1 MBC h-1)
April
July
Sampling season

◀**Fig. 1** Changes in **a** net N mineralization and **b** nitrification rates, **c** microbial biomass carbon (*MBC*) and **d** nitrogen (*MBN*), **e** basal respiration (*BR*), **f** extractable dissolved organic C (*DOC*), **g** microbial quotient (*qMIC*), and **h** microbial metabolic quotient (qCO_2) in surface (0–15 cm) soil in the control, nitrogen (*N*), phosphorus (*P*), and both nitrogen and phosphorus (*N* + *P*)-treated plots. *Bars* represent standard errors (n=6). *Above the bars*, the superscript letters "N" and "S" indicate a significant effect of N fertilization or sampling season, respectively, and "S × N" indicates an interactive effect, followed by the level of statistical significance (based on repeated measures ANOVA, with N and P addition treatments as between-subject effects and sampling season as within-subject effect)

ly). Moreover, the interaction of P with N addition did not significantly affect MBC and *q*MIC (P=0.326 and P=0.820, respectively; Fig. 1c, g). There was no effect of sampling season on MBC and *q*MIC (Fig. 1c, g). Nitrogen addition significantly decreased MBN (P=0.004) and its interaction with sampling season affected MBN (P=0.016; Fig. 1d). No significant effects of N addition (P=0.958), P addition (P=0.121), and their interactions (P=0.269) on BR were detected (Fig. 1e). Therefore, N addition significantly increased qCO_2 (P=0.001) due to the significant decrease of MBC with N addition, but P addition (P=0.082) and its interaction with N addition (P=0.061) did not significantly affect qCO_2 (Fig. 1h). In addition, N addition significantly (P=0.010) increased extractable DOC (Fig. 1f).

Discussion

Our results show that N addition decreased MBC and MBN (Fig. 1c, d), which confirms what was observed in temperate grassland soils (Sarathchandra et al. 2001; Treseder 2008). Sarathchandra et al. (2001) suggested that the decline in MBC by N addition was related to the reduction in SOC, which is the energy source of soil microorganisms. In our study, however, SOC was not affected by N addition over 5 years (Table 1) even though C input was significantly increased due to the increased aboveground biomass (Zeng et al. 2010). The cause of decreased MBC is probably due to the soil acidification by nitrification caused by N addition (Table 1); indeed, accumulation of NO_3^-–N was observed (Fig. 1b). Aciego Pietri and Brookes (2009) suggested that soil pH is an important factor controlling biomass, activity, and community structure of soil microorganisms. A recent study also showed marked influence of pH on growth and biomass of soil microorganisms and composition of decomposers (Rousk et al. 2009). Low pH can decrease microbial activities and growth of soil microorganisms (Rousk et al. 2009) and consequently reduce microbial biomass under N addition (Fig. 1c, d).

The lack of effects of N addition on soil respiration with the decline in microbial biomass (Fig. 1c–e) does not agree with what was reported by Treseder (2008). The decrease in microbial biomass was responsible for the increase in qCO_2 and the decline in *q*MIC values (Fig. 1g, h). Probably, despite the significant increase in extractable DOC under N addition (Fig. 1f), there was a decline in the efficiency of converting available C into microbial biomass.

In our investigation, P addition had negligible effects on soil net N mineralization and nitrification rates, MBC, MBN, BR, and extractable DOC, and thus on *q*MIC and qCO_2 values, although it significantly increased soil TP concentration (Table 1). Moreover, P addition had no significant interactions with N addition on these soil properties, probably due to the negligible effects of P addition on plant N uptake associated with negligible effects on plant growth (unpublished data).

In conclusion, N addition over 5 years did not affect SOC, TN, and TP concentrations in semi-arid, sandy grassland. Nitrogen addition decreased soil pH which probably reduced microbial biomass, and thus declined *q*MIC and increased qCO_2 values, while it also increased net nitrification rate and extractable DOC concentration. However, P addition had no effects on soil microbial properties. These results suggest that in the mid-term the high input of N may decrease microbial biomass due to induced pH effects, with a possible decline in soil quality.

Acknowledgments This work was funded by the National Key Basic Research Program of China (no. 2007CB106803) and the National Key Technologies R&D Program of China (nos. 2006BAD26B0201-1 and 2006BAC01A12). We thank Dr. Paolo Nannipieri and two anonymous reviewers for their valuable comments and suggestions, which greatly helped improve the manuscript. We are also grateful to Gui-Yan Ai, He-Ming Lin, Jing-Shi Li, and Yun-Xia Liu for laboratory analyses.

References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. Bioscience 39:378–386

Aciego Pietri JC, Brookes PC (2009) Substrate inputs and pH as factors controlling microbial biomass, activity and community structure in an arable soil. Soil Biol Biochem 41:1396–1405

Bennett LT, Adams MA (2001) Response of a perennial grassland to nitrogen and phosphorus additions in sub-tropical, semi-arid Australia. J Arid Environ 48:289–308

Cabrera ML, Beare MH (1993) Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci Soc Am J 57:1007–1012

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142

Filip Z (2002) International approach to assessing soil quality by ecologically-related biological parameters. Agric Ecosyst Environ 88:169–174

Hu YL, Wang SL, Zeng DH (2006) Effects of single Chinese fir and mixed leaf litters on soil chemical, microbial properties and soil enzyme activities. Plant Soil 282:379–386

Insam H, Parkinson D, Domsch KH (1989) Influence of macroclimate on soil microbial biomass. Soil Biol Biochem 21:211–221

Jenkinson DS (1988) Determination of microbial biomass carbon and nitrogen in soil. In: Wilson JR (ed) Advances in nitrogen cycling in agricultural ecosystems. CAB International, Wallingford, pp 368–386

Kaschuk G, Alberton O, Hungria M (2010) Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. Soil Biol Biochem 42:1–13

Li FR, Zhao LY, Zhang H, Zhang TH, Shirato Y (2004) Wind erosion and airborne dust deposition in farmland during spring in the Horqin Sandy Land of eastern Inner Mongolia, China. Soil Tillage Res 75:121–130

Lü C, Tian H (2007) Spatial and temporal patterns of nitrogen deposition in China: synthesis of observational data. J Geophys Res 112:D22S05. doi:10.1029/2006JD007990

Mahowald N, Jickells TD, Baker AR, Artaxo P, Benitez-Nelson CR, Bergametti G, Bond TC, Chen Y, Cohen DD, Herut B, Kubilay N, Losno R, Luo C, Maenhaut W, McGee KA, Okin GS, Siefert RL, Tsukuda S (2008) Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. Global Biogeochem Cycles 22:GB4026. doi:10.1029/2008GB003240

Malý S, Královec J, Hampel D (2009) Effects of long-term mineral fertilization on microbial biomass, microbial activity, and the presence of *r*- and *K*-strategists in soil. Biol Fertil Soils 45:753–760

Masto RE, Chhonkar PK, Singh D, Patra AK (2008) Alternative soil quality indices for evaluating the effect of intensive cropping, fertilisation and manuring for 31 years in the semi-arid soils of India. Environ Monit Assess 136:419–435

Matson P, Lohse KA, Hall SJ (2002) The globalization of nitrogen deposition: consequences for terrestrial ecosystems. Ambio 31:113–119

Menyailo OV, Lehmann J, Cravo MD, Zech W (2003) Soil microbial activities in tree-based cropping systems and natural forests of the Central Amazon, Brazil. Biol Fertil Soils 38:1–9

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. Eur J Soil Sci 54:655–670

Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (eds) Methods of soil analysis. Part 3. Chemical methods. Soil Science Society of America Book Series, Wisconsin, pp 961–1010

Robertson GP, Wedin D, Groffman PM, Blair JM, Holland EA, Nadelhoffer KJ, Harris D (1999) Soil carbon and nitrogen availability: nitrogen mineralization, nitrification and carbon turnover. In: Robertson GP, Bledsoe CS, Coleman DC, Sollins P (eds) Standard soil methods for long term ecological research. Oxford University Press, New York, pp 258–271

Rousk J, Brookes PC, Bååth E (2009) Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Appl Environ Microbiol 75:1589–1596

Sarathchandra SU, Ghani A, Yeates GW, Burch G, Cox NR (2001) Effect of nitrogen and phosphorus fertilizers on microbial and nematode diversity in pasture soils. Soil Biol Biochem 33:953–964

Schoumans OF, Groenendijk P (2000) Modeling soil phosphorus levels and phosphorus leaching from agricultural land in the Netherlands. J Environ Qual 29:111–116

Stöcklin J, Schweizer K, Körner C (1998) Effects of elevated CO_2 and phosphorus addition on productivity and community composition of intact monoliths from calcareous grassland. Oecologia 116:50–56

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol Lett 11:1111–1120

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. Soil Biol Biochem 19:703–707

Vitousek PM, Aber J, Howarth RW, Likens G, Matson P, Schindler D, Schlesinger W, Tilman D (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7:737–750

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soils. Biol Rev 67:321–358

Wardle DA, Ghani A (1995) A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biol Biochem 27:1601–1610

Zeglin LH, Stursova M, Sinsabaugh RL, Collins SL (2007) Microbial responses to nitrogen addition in three contrasting grassland ecosystems. Oecologia 154:349–359

Zeng DH, Li LJ, Fahey TJ, Yu ZY, Fan ZP, Chen FS (2010) Effects of nitrogen addition on vegetation and ecosystem carbon in a semi-arid grassland. Biogeochemistry 98:185–193