Nitrogen addition alters elemental stoichiometry within soil aggregates in a temperate steppe

Jinfei Yin [1,2], Ruzhen Wang [1,*], Heyong Liu [1], Xue Feng [1], Zhuwen Xu [1], Yong Jiang [1]

[1] State Engineering Laboratory of Soil Nutrient Management, Institute of Applied

Ecology, Chinese Academy of Sciences, Shenyang 110016, China

[2] University of Chinese Academy of Sciences, Beijing 100049, China

Correspondence to: Ruzhen Wang (ruzhenwang@iae.ac.cn)

Abstract

Ongoing increases in anthropogenic nitrogen (N) inputs have largely affected soil carbon (C) and nutrients cycling in most terrestrial ecosystems. Numerous studies have concerned the effects of elevated N inputs on soil dissolved organic carbon (DOC), dissolved inorganic N (DIN), available phosphorus (AP), exchangeable calcium (Ca) and magnesium (Mg), and available iron (Fe) and manganese (Mn). However, little has emphasized on stoichiometric traits of these soil parameters, especially within different soil aggregate fractions. In a semi-arid grassland of Inner Mongolia, we studied effect of N addition on the ratios of DOC:DIN, DOC:AP, DIN:AP, exchangeable Ca:Mg, available Fe:Mn within three soil aggregate classes of large macroaggregates (> 2000 μm), small macroaggregates (250-2000 μm), and microaggregates (< 250 μm). Elevated N inputs significantly decreased DOC:DIN ratio within three soil aggregates. Soil DOC:AP ratio significantly decreased along with increasing N gradients within large macro- and microaggregates. Nitrogen significantly decreased the ratio of exchangeable Ca:Mg within soil macroaggregates. The ratio of available Fe:Mn decreased with N addition within three soil aggregate classes. Alteration of elemental stoichiometry within soil fractions that are featured by different nutrient retention capacity will influence chemical composition of soil microorganisms and plant quality.

Key words: nitrogen deposition, soil fraction, C:N:P stoichiometry, Ca:Mg ratio, Fe:Mn ratio

Introduction

   Stoichiometric relationships of soil nutrients are reliable indicators of nutrient availabilities for plants and soil microorganisms (Cleveland and Liptzin, 2007). Soil carbon:nitrogen:phosphorus (C:N:P) ratios play essential roles in shaping plant and microbial stoichiometry which reveals nutrient allocations, life history strategies, and physiological adjustment to climate change (Elser et al., 2010). For instance, enhanced N inputs resulted in higher soil N availability (Wei et al., 2013) and consequentially decrease of foliar C:N and increase of foliar N:P ratio (Han et al., 2014). Nitrogen addition was suggested to cause divergent effects on plant C:P ratio which declined at species-level but showed no change at the community-level (Han et al., 2014). Plant species that are stoichiometric N:P flexibility may have advantage over these possessing strict stoichiometric homeostasis under environmental changes (Sardans and Pe ñuelas, 2012). Leaves with high nutrient concentrations (both N and P), which is relatively lower C:N and C:P ratios tend to be short lived with high specific leaf area (Wright et al., 2004) as well as high photosynthetic capacity and dark respiration rates (Reich et al., 2008). Local nutrient availability from soils is suggested to be one of the most important contributors to the wide variation of terrestrial plants in C:N:P ratios (i.e. plant are what they root in) (Elser, 2010). Soil exchangeable calcium (Ca) and magnesium (Mg) and available iron (Fe) and manganese (Mn) are critical metal nutrients for plant growth, microbial activity, and ecosystem health (Lucas et al., 2011). The ratio of exchangeable Ca:Mg indicates the relative availability of these two ions and is reported to influence clay dispersion and

surface sealing processes (Dontsova and Norton, 2002). Maintaining proper available

Fe:Mn ratio is essential for plant health as lower ratio might suggest plants suffering

from Fe deficiency and Mn toxicity (Hodges, 2010). However, the stoichiometric

qualification of soil DOC and available nutrients is less known than plant

5   stoichiometry.

Ongoing increases in anthropogenic N inputs have largely affected soil C and

nutrient cycles in most terrestrial ecosystems (Lü et al., 2013; Neff et al., 2002).

Under N enrichment, higher soil available N (sum of nitrate and ammonium) has been

documented to be positively (Wei et al., 2013), negatively (Heffernan and Fisher,

10   2012) or even neutrally (Liu and Greaver, 2010) related to dissolved organic C (DOC),

which is an important linkage for plant-soil-microbe system in natural ecosystems.

Anthropogenic increases in reactive N would also shift ecosystem nutrient limitation

from N to P by stimulating P uptake by plants (Menge and Field, 2007). Moreover,

soil acidification caused by N enrichment could help desorption and dissolution of

15   soil inorganic P serving as an essential P source for plants, especially in calcareous

soils (Lajtha and Bloomer, 1988; Tunesi et al., 1999). Soil pH is widely recognized as

one of the most influential factors that regulates nutrient bioavailability (Kemmitt et

al., 2006). For example, Piccolo (2001) suggested that drop of soil pH elevated

solubility of soil humic substances. Soil pH is also suggested to influence soil C:N

20   ratio via changes in plant litter quality (Schmidt, 1982) and increase soil available P

(AP) through promoting phosphomonoesterase activity (Hogan et al., 2010). Thus, N

enrichment might influence soil available C:N:P stoichiometry through the alteration

of soil pH, and hence aggravate or alleviate plant and microbial C and nutrient

limitations (Elser, 2010). Enhanced N inputs causes leaching of soil exchangeable Ca

and Mg and activation of soil available Fe and Mn as a result of soil acidification

which will lead to nutrient imbalance in soils (Katou, 2002; Malhi et al., 1998). For

5   example, N deposition decreases the ratio of exchangeable Ca:Mg as preferential

weathering of soil exchangeable Ca relative to Mg (Lu et al., 2014). Iron deficiency

chlorosis has been found in calcareous soils in grassland of America (Rogovska et al.,

2007) and Mn toxicity may occur after soil acidification companying with leaching of

base cations (Lynch and Clair, 2004). Thus, available Fe:Mn ratio will be useful in

10   indicating relative availability of Fe and Mn as affected by N addition. Under

anthropogenic N enrichment scenarios, clarifying stoichiometric traits of soil

available elements would promote our understanding of soil nutrient imbalance as

caused by environmental changes.

Physical protection by soil aggregation is a main mechanism of soil organic

15   matter (SOM) stabilization (Wiesmeier, 2012). Oxygen and water diffusion rates is

generally parallel with soil aggregate sizes as being determined by pore space (Young

and Ritz, 2000; Baker et al., 2007). Consequently, available nutrients, like N and P

tend to distribute in aggregates of larger size as more favorable microclimate for

microbial activity herein (Dorodnikov et al., 2009; Wu, 2012). Smaller aggregate-size

20   classes are confirmed as a preferential site for the physical stabilization of SOM and

nutrient retention (Fonte et al., 2014). Higher clay contents in microaggregates tend to

provide more surface area and binding sites to retain more exchangeable Ca and Mg

(Oorts et al., 2003). Within microaggregates, more microbial-processed SOM will also chelate or complex larger amount of base cations and micronutrients (Lü et al., 2016). Even though numerous studies have focused on C and nutrients cycling in aggregate scale (Fonte et al., 2014; Six and Paustian, 2014; Wright, 2009),

5 stoichiometric traits of DOC and available nutrients within aggregates are still unclear.

Semiarid steppes of Inner Mongolia in northern China, playing a vital role in serving environmental health, regional economy, and the global C cycle, have undergone degradation during the last 50 years' increased stocking rates and static

10 grazing management practices (Kang et al., 2007). Non-physically protected SOM pools, which can be a majority of SOC and nutrients in these sandy grassland systems, are however, inherently vulnerable to environmental changes (Creamer et al., 2011), like elevated N inputs. A 7-year N field manipulation experiment has already showed significant aboveground changes in species turnover rates, plant community

15 composition, and community stability (Xu et al., 2012). Microaggregates (< 250 μm) had the highest dissolved inorganic N (DIN) concentration as compared to large ($>$ 2000 μm) and small macroaggregates (250-2000 μm) (see Wang et al., 2015a) which was not the case for both DOC (see Wang et al., 2015b) and AP (Wang et al., 2016). Nitrogen addition significantly increased DIN (see Wang et al., 2015a) and available

20 Fe and Mn but decreased exchangeable Ca and Mg across three soil fractions (not for Ca in microaggregates) (Wang et al., 2017). We analyzed stoichiometric traits of DOC, DIN, AP, exchangeable Ca and Mg, and available Fe and Mn within soil aggregates in

this semi-arid grassland. We hypothesize that microaggregates had lower DOC:DIN

and higher DIN:AP ratios as better N retention capacity in smaller-size aggregates.

We also hypothesized that N addition would decrease DOC:DIN while increase

DOC:AP and DIN:AP ratios as higher anthropogenic inputs of inorganic N and

5   intensive removal of available P from soil by plant uptake. As preferential weathering

of Ca relative Mg during soil acidification, we expected to detect significant decrease

of Ca:Mg ratio under N addition across soil aggregate classes. Due to the fact that

plant growth is commonly limited by Fe deficiency in calcareous grasslands,

enhanced Fe translocation from soil to plants would result in lower soil available

10   Fe:Mn ratio under N enrichment.

Methods

Study site and experimental design

The study site (42°02′27″N, 116°17′59″E, elevation 1,324 m a.s.l) was located in

15   Duolun County, a semi-arid area in Inner Mongolia, China. Mean annual temperature

is 2.1 ℃, ranging from 17.8 ℃ in January to 18.8 ℃ in July and average precipitation is

379.4 mm yr^{-1}. The soil is a chestnut in Chinese classification and Calcisorthic

Aridisol in the U.S. Soil Taxonomy classification. Soil texture of the experimental site

is sandy loam with 62.75 % sand, 20.30 % silt, and 16.95 % clay (Liu et al. 2009).

20   In April 2005, seven blocks (107 m $\times$ 8 m) were set up in a split-plot design with

water and N being the two treatments. Each block was divided into two main plots

receiving either ambient precipitation or additional water treatment (180 mm). Each

main plot was divided into six subplots which were randomly treated with four levels of N (0 [CK], 5 [N_5], 10 [N_{10}], and 15 [N_{15}] g N m^{-2} yr^{-1}), P (10 g N m^{-2} yr^{-1}), and combined N and P addition. This study only concerned about the treatments of four levels of N addition under ambient precipitation. Nitrogen was added as urea pellets

5 with half of it applied in early May and the other half in late June.

Field sampling and measurements

  Soil Samples (0-10 cm) were collected from each N plot from four out of seven blocks on August 2013. For each plot, five random cores were retrieved after removing litter layer and stored at 4 ℃ under further analysis. Soil aggregates were

10 isolated according to Dorodnikov et al. (2009) into three aggregates (> 2mm, large macroaggregates class; 2-0.25 mm, small macroaggregates; and < 0.25mm, microaggregates class) by a Retsch AS 200 Control (Retsch Technology, Düsseldorf, Germany). This dry-sieving method was used to maintain microbial activity and reduce the loss of DOC and available nutrients in soil aggregates. The separated

15 aggregates were weighed and used for determining soil aggregate moisture (drying at 105 ℃ for 48 h), DOC, DIN, AP, exchangeable Ca and Mg, and available Fe and Mn.

  Soil pH was measured in a 1:2.5 (w/v) soil-to-water slurry of soil aggregate samples with a digital pH meter. The concentration of DOC was extracted by adding 50 mL of 0.5 M potassium sulfate and analyzed by a TOC analyzer (High TOC,

20 Elementar). Soil DIN concentration was extracted with 50 mL 2 M KCl from 10 g fresh soils (McLeod, 1992) and determined colorimetrically using an AutoAnalyser III continuous Flow Analyzer (Bran & Luebbe, Norderstedt, Germany). Soil AP was

measured using the Olsen method (Olsen et al., 1954). Briefly, 2.5 g of the soil

fraction was mixed with 50 mL 0.5 M $NaHCO_3$ (pH 8.5) and 5 g of phosphorus-free

charcoal. The mixture was shaken at 150 rpm for 30 min and filtered. The P

concentration of P in filtration was determined by molybdenum blue colorimetric

5 method (Murphy and Riley, 1962). The units of DOC, DIN and AP were expressed in

mg per kg soil aggregate. Exchangeable Ca and Mg within soil aggregates were

extracted by mixing 2.5 g soil fraction with 50 mL 1M ammonium acetate (pH 7.0)

and the shaken for 30 min at 150 rpm. After filtration, the Ca and Mg concentrations

in extraction were determined by atomic absorption spectrometer (AAS, Shimadzu,

10 Japan). To analyze available Fe and Mn within soil aggregate fractions, 10 g of soil

samples was extracted by 20 mL 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA

(triethanolamine) (pH 7.0). After shaken at 180 rpm for 2 h, the extracts were filtered

and available Fe and Mn concentrations in the filtration were determined by the AAS.

Soil DOC and available nutrients of control plot was listed in Table 1. The

15 concentrations of exchangeable Ca and Mg were represented as cmol per kg soil

aggregate and it was mg per kg soil aggregate for available Fe and Mn.

Statistical analysis

The normality of data and homogeneity of variances were determined by

Kolmogorov-Smirnov test and Levene's test, respectively. Two-way ANOVAs were

20 executed to determine soil aggregate size (S), N addition, and their interactive effects

(S ×N) on the ratios of DOC:DIN, DOC:AP, DIN:AP, exchangeable Ca:Mg, and

available Fe:Mn. Pearson correlation analysis was used to determine the relationship

between soil pH and these ratios within each soil aggregate classes. All the correlation analysis was performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, U.S.A.) and statistical significance was accepted at P<0.05.

5   **Results**

N addition effects on soil available C:N:P

The ratio of DOC:DIN varied from 1.4 to 3.4 within large macroaggregates, from 1.0 to 1.5 within small macroaggregates, and from 1.0 to 1.3 within microaggregates (Fig. 1a). The DOC:DIN ratio was significantly higher in large macroaggregates than

10   in both small macro- and microaggregates averaging across all N treatments (Fig. 1a). Nitrogen addition significantly decreased DOC:DIN ratio within three soil aggregate classes (Fig. 1a). Significant interaction between N and aggregate size (S) was detected on DOC:DIN ratio (Table 2).

No difference in soil DOC:AP ratio was found among three aggregate classes (Fig.

15   1b). Soil DOC:AP ratio significantly decreased with N addition in both large macro- and microaggregates, but it increased under N_5 in small macroaggregates (Fig. 1b). No significant interactive N×S was detected on DOC:AP ratio (Table 2).

The DIN:AP ratio was significantly lower in large macroaggregates as compared to both small macro- and microaggregates (Fig. 1c). Soil DIN:AP ratio showed no

20   change along N gradients (Fig. 1c). There was a significant interaction of N×S on the DIN:AP ratio (Table 2).

Nitrogen addition effects on exchangeable Ca:Mg and available Fe:Mn ratios

The exchangeable Ca:Mg ratio ranged from 11.0 to 15.0 across soil aggregate

classes (Fig. 2a). Nitrogen addition significantly decreased Ca:Mg ratio within large

and small macroaggregates (Fig. 2a). Neither aggregate size nor interactive N and

aggregate size effects was detected on changes of Ca:Mg ratio.

5   The ratio of available Fe:Mn ranged from 0.98 to 1.57 across three soil aggregate

fractions (Fig. 2b). No difference in Fe:Mn ratio was detected among three soil

fractions (Fig. 2b, Table 2). Nitrogen addition significantly increased soil available

Fe:Mn within three soil fractions (Fig. 2b). Nitrogen addition significantly interacted

with soil aggregate size to affect the ratio of available Fe:Mn (Table 2).

10 *Correlation analyses*

The DOC:DIN ratio was positively correlated with soil pH within three soil

fractions (Fig. 3a,b,c). Significantly positive relationship was found between DOC:AP

ratio and soil pH within large macro- and microaggregates (Fig. 3d,f). Significant and

negative correlation between DIN:AP ratio and soil pH was only detected in small

15 macroaggregates (Fig. 3h). Within large and small macroaggregates, the Ca:Mg ratio

was significantly and positively correlated with soil pH (Fig. 4a,b). Significant and

negative relationships between the Fe:Mn ratio and soil pH were found within three

soil fractions (Fig. 4d,e,f).

20 **Discussion**

The stoichiometric ratios among different soil aggregates fractions

Consistent with our initial hypothesis, microaggregates had lower DOC:DIN ratio

and higher DIN:AP ratio relative to large macroaggregates. It's generally recognized

that more labile SOM concentrates in macroaggregates while more recalcitrant SOM

in microaggregates (Jastrow et al., 2007). Additionally, SOM in microaggregates

experiences more microbial processing cycles which is more decomposed as

5   compared to macroaggregates (Six and Paustian, 2014). These resulted in lower C:N

of SOM in microaggregates. Our previous study suggested that microaggregates

retained significantly higher inorganic N relative to large macroaggregates (Wang et

al., 2015a). However, DOC and AP concentrations showed no difference between

large macro- and microaggregates as suggested by our previous studies (Wang et al.,

10   2015b, 2016). In this context, microaggregates had significantly lower DOC:DIN

ratio (Fig. 1a) but higher DIN:AP ratio (Fig. 1c) than large macroaggregates. Our

results indicated that microaggregates were preferential sites for retaining DIN

relative to DOC and AP as compared to large macroaggregates (Fig. 1a,c).

Microaggregates serve as an indicator of soil C storage capacity and help elucidate the

15   capacity of soils to protect SOM and supply N and P nutrients (Du et al., 2014).

Higher DIN:AP ratio in microaggregates would be essential for available N supply,

but it might eventually result in P deficiency under enhanced N loading rates in the

long term.

Effect of N addition on DOC:DIN, DOC:AP, and DIN:AP ratios within soil

20   *aggregates*

       As expected, we observed a significant decrease of soil DOC:DIN ratio within all

soil aggregate classes as affected by N addition (Fig. 1a). This should mainly be due

to supply of DIN which is directly transformed from urea fertilizer or indirectly

derived from enhanced soil organic N mineralization and nitrification under urea

addition (Chen et al., 2013) within three soil fractions. Indeed, our previous studies

suggested that N addition increased DIN by up to 128.3%, 41.2%, and 45.7% (Wang

5   et al., 2015a), while DOC only by 12.7%, 3.7%, and 12.7% within large macro-, small

macro-, and microaggregates, respectively (Wang et al., 2015b). As suggested by our

previous work, N addition decreased soil pH from 7.3 to 5.6 in large macroaggregates,

from 7.5 to 5.8 in small macroaggregates, and from 7.4 to 5.9 in microaggregates

(Wang et al., 2015b). Soil pH was one of the main factors influencing DOC:DIN ratio

10   as suggested by their positive correlations within three soil fraction (Fig. 3a,b,c).

Decline of soil pH was reported to be associated with accumulation of soil DIN

(Aciego Pietri and Brooks, 2008), thus leading to the decrease of DOC:DIN ratio (Fig.

3a,b,c).

Soil DOC and DIN are important energy and N sources for microorganisms, and

15   relative soil C and N availabilities (represented as DOC:DIN) make great contribution

to controlling microbial activity and nutrient cycling processes (Dijkstra et al., 2006;

Wu et al., 2012). Significant decrease of DOC:DIN (Fig. 1a) might suggest that higher

N inputs induced microbial C limitation relative to N across soil fractions. However,

Wei et al. (2013) found that N addition increased both DOC and DIN but decreased

20   microbial biomass C (MBC) concentration; and they suggested that soil microbes

were no longer C-limited as non-significant correlation between DOC and microbial

biomass C. In their study, they failed to analyze the relationship between DOC:DIN

ratio and MBC which could be used to indicate relative C and N availabilities in determining microbial growth. In this study, coincident decrease of DOC:DIN ratio (Fig. 1a) and MBC (see Wang et al., 2015b) indicated that repression of microbial growth under N addition was due to lower C availability relative to N (Treseder, 2008).

Our previous study suggested that N addition increased soil AP by 60.2% and 84.5% within large macro- and microaggregates, respectively (Wang et al., 2016). Based on this, it was reasonable to detect significant decrease of the ratio of DOC:AP within large macro- and microaggregates (Fig. 1b). Soil acidification could enhance dissolution of phosphate from mineral-bound P pools while it was not the case for DOC (Wang et al., 2016). Thus, production of DOC might not keep up with continuous dissolution of AP resulting in lower DOC:AP ratio under N addition (Fig. 1b). Significant positive correlations between soil pH and DOC:AP ratio (Fig. 3d,f) also confirmed higher AP dissolution than DOC supply under soil acidification. Under the scenario of enhanced ecosystem N inputs, decrease of soil aggregate DOC:AP ratio would induce microbial C limitation relative to P (Treseder, 2008), and continuous release of AP from rock weathering would shift the ecosystem from N limitation to P limitation in the long term (Lü et al., 2013).

Effect of N addition on exchangeable Ca:Mg and available Fe:Mn ratios

Within large and small macroaggregates, significant decrease of exchangeable Ca:Mg ratio supported our hypothesis. Soil acidification, as developed under increased SO_2 and NO_x emissions, results in weathering and release of base cations

(Lucas et al., 2011). Selective weathering of certain base cations (Ca > Na > Mg > K) would result in change of base cation budgets and imbalance of metal ions in soils (Lu et al., 2014). Preferential loss of exchangeable Ca relative to Mg was the main reason of significant decrease of Ca:Mg ratio in large and small macroaggregates (Fig. 2a).

5   Correlation analyses also suggested coincident decline of Ca:Mg ratio and soil pH indicating that more exchangeable Ca was leached than Mg during soil acidification in macroaggregates. However, unaffected Ca:Mg ratio in microaggregates declined our hypothesis that Ca:Mg ratio would decrease across three soil fractions. In this sandy grassland, microaggregates, containing more minerals and less sand would be a

10   more favorable site for base cation binding and adsorption which was less affected by N addition and sequential soil acidification comparing to macroaggregates (Gunina and Kuzyakov, 2014). Moreover, more decomposed SOM as indicated by $\delta^{13}C$ (Wang et al., 2015a) would provide additionally strong binding surface for exchangeable Ca and Mg in microaggregates. Thus, significant relationships of Ca:Mg ratio with soil

15   pH was not detected in microaggregates (Fig. 4c).

Soil Ca:Mg ratio affects plant Cu uptake and lower Ca:Mg might increase or decrease plant Cu uptake and toxicity depending on plant species and soil types (Lombini et al., 2003). Under enhanced N inputs, significant decrease of Ca:Mg ratio would further influence nutrient balance, i.e. Cu uptake of plants in this calcareous

20   grassland. Researchers proposed that relative availabilities of exchangeable Ca and Mg were more important that their absolute amounts (Bear et al., 1951; Albrecht, 1975; Kopittke and Menzies, 2007). And optimal Ca:Mg ratio ranges for plant growth

were identified for various plant species since the study from Loew (1892). In

addition, different soil aggregate classes were featured by their various nutrient supply

capacities (Mikha and Rice, 2004). Soil structure could modify the effect of cation

ratios on plant growth (Zhang and Norton, 2002). These shed light on the essential

5   role of studying soil exchangeable Ca:Mg ratio of different soil aggregate fractions in

evaluating soil fertility and plant growth.

For all soil fractions, significant increase of available Fe:Mn ratio denied our

initial hypothesis. Decrease of the Fe:Mn ratio might be caused the antagonistic

relationship between Fe and Mn during plant uptake (Somers and Shive, 1942). Soil

10   acidification promoted weathering and desorption of micronutrients from soil

minerals which increased soil available Fe and Mn concentrations (Malhi et al., 1998).

Even though Fe availability generally limits productivity in calcareous grasslands,

enhanced plant Mn uptake might retard plant Fe acquisition leading to higher

available Fe retained in soil (Tanaka and Navasero, 1966; Tian et al., 2016) under soil

15   acidification. Correspondingly, negative relationships of soil pH with Fe:Mn ratio (Fig.

4d,e,f) suggested accumulation of available Fe relative to Mn under soil acidification

as affected by enhanced N inputs. Consistent with our findings, Tian et al. (2015 and

2016) found soil acidification enhanced Mn uptake and reduced Fe absorption by

forbs which result in lower photosynthetic rates and growth and loss of forb species.

20   Plant Fe deficiency was reported to induce chlorosis and decrease photosynthesis in

calcareous grassland (Rogovska et al., 2007). Increase of soil available Fe:Mn ratio

might pose threat to grassland productivity as potential aggravation of Fe deficiency

and occurrence of Mn toxicity to plant species (Tian et al., 2016). Soil nutrient imbalance would not only influence both quantity (yield) and quality of plants, but also cause impact on health of animals grazing on these plants (Mikha and Rice, 2004).

Conclusion

After 9-year N amendment, we found that microaggregates had lower DOC:DIN but higher DIN:AP ratio. The soil DOC:DIN ratio decreased with N addition within three fractions as a result of exogenous N supply. Nitrogen addition decreased DOC:AP

10 ratio in large macro- and microaggregates which might induce microbial C limitation relative to P. Nitrogen addition significantly decreased exchangeable Ca:Mg ratio within macroaggregates which purportly due to preferential leaching of exchangeable Ca under soil acidification as developed with N addition. Within three soil aggregate classes, significant increase of available Fe:Mn ratio might cause plant Fe deficiency

15 and Mn toxicity as affected by ecosystem N enrichment. Changes in the ratios of available elements among soil fractions that are responsible for different nutrient retention and cycling processes will cause nutrient imbalance, affect plant and microbial nutrient acquisition and chemical composition, and influence ecosystem productivity and health.

Author contribution

Y. Jiang and Z. Xu designed the experiment; J. Yin, H. Liu, and R. Wang carried them

out. X. Feng helped to do the laboratory analyses. R. Wang prepared the manuscript with contributions from all authors.

Acknowledgements

5 This work was financially supported by the National Key Research and Development Program of China (2016YFC0500707) and the National Natural Science Foundation of China (41371251).

References

10 Aciego Pietri, J. C., and Brookes, P.C.: Nitrogen mineralisation along a pH gradient of a silty loam UK soil. Soil Biol. Biochem., 40, 797-802, 2008.

Albrecht, W. A.: The Albrecht papers. Vol. 1: Foundation concepts. Acres USA, Kansas City, 1975.

Baker, J. M., Ochsner, T. E., Venterea, R. T., and Griffis, T. J.: Tillage and soil carbon

15 sequestration - What do we really know? Agr. Ecosyst. Environ., 118, 1-5, 2007.

Bear, F. E., Prince, A. L., Toth, S. J., and Purvis, E. R.: Magnesium in plants and soil. Bull. 760. New Jersey Agric. Exp. Stn., New Brunswick, 1951.

Chen, Y., Xu, Z., Hu, H., Hu, Y., Hao, Z., Jiang, Y., and Chen, B.: Responses of ammonia-oxidizing bacteria and archaea to nitrogen fertilization and precipitation

20 increment in a typical temperate steppe in Inner Mongolia. Appl. Soil Ecol., 68, 36-45, 2013.

Cleveland, C.C., and Liptzin, D.: C:N:P stoichiometry in soil: is there a "Redfield

ratio" for the microbial biomass? Biogeochemistry 85, 235–252, 2007.

Creamer, C. A., Filley, T. R., Boutton, T. W., Oleynik, S., and Kantola, I. B.: Controls on soil carbon accumulation during woody plant encroachment: Evidence from physical fractionation, soil respiration, and $\delta^{13}C$ of respired CO_2. Soil Biol.

5   Biochem., 43, 1678-1687, 2011.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S. C., Schwartz, E., Menyailo, O. V., and Hungate, B. A.: ^{13}C and ^{15}N natural abundance of the soil microbial biomass. Soil Biol. Biochem., 38, 3257-3266, 2006.

Dontsova, K. M., and Norton, L. D.: Clay dispersion, infiltration, and erosion as

10   influenced by exchangeable Ca and Mg. Soil Science, 167, 184-193, 2002.

Dorodnikov, M., Blagodatskaya, E., Blagodatsky, S., Marhan, S., Fangmeier, A., and Kuzyakov, Y.: Stimulation of microbial extracellular enzyme activities by elevated CO_2 depends on soil aggregate size. Global Change Biol., 15, 1603-1614, 2009.

Du, Z., Ren, T., Hu, C., and Zhang, Q.: Transition from intensive tillage to no-till

15   enhances carbon sequestration in microaggregates of surface soil in the North China Plain. Soil Till. Res., 146, 26-31, 2015.

Elser, J. J., Fagan, W. F., Kerkhoff, A. J., Swenson, N. G., and Enquist, B. J., Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. New Phytol., 186, 593-608, 2010.

20   Fonte, S. J., Nesper, M., Hegglin, D., Velásquez, J. E., Ramirez, B., Rao, I. M., Bernasconi, S. M., Bünemann, E. K., and Frossard, E.: Pasture degradation impacts soil phosphorus storage via changes to aggregate-associated soil organic matter in

highly weathered tropical soils. Soil Biol. Biochem., 68, 150-157, 2014.

Gunina, A., and Kuzyakov, Y.: Pathways of litter C by formation of aggregates and SOM density fractions: implications from ^{13}C natural abundance. Soil Biol. Biochem., 71, 95-104, 2014.

5   Heffernan, J. B., Fisher, S. G.: Plant-microbe interactions and nitrogen dynamics during wetland establishment in a desert stream. Biogeochemistry, 107, 379–391, 2012.

Hodges, S. C.: Soil fertility basics. Soil Science Extension, North Carolina State University, 2010.

10   Hogan, E. J., Minnullina, G., Smith, R. I., Crittenden, P. D.: Effects of nitrogen enrichment on phosphatase activity and nitrogen: phosphorus relationships in Cladonia portentosa. New Phytol., 186: 911-925, 2010.

Jastrow, J. D., Amonette, J. E., and Bailey, V. L.: Mechanisms controlling soil carbon turnover and their potential application for enhancing carbon sequestration. Clim.

15   Change, 80, 5-23, 2007.

Kang, L., Han, X., Zhang, Z., and Sun, O. J.: Grassland ecosystems in China: review of current knowledge and research advancement. Philos. Trans. R. Soc. B., 362, 997-1008, 2007.

Kemmitt, S. J., Wright, D., Goulding, K. W., Jones, D. L.: pH regulation of carbon

20   and nitrogen dynamics in two agricultural soils. Soil Biol. Biochem., 38, 898-911, 2006.

Katou, H.: A pH-dependence implicit formulation of cation-and anion-exchange

capacities of variable-charge soils. Soil Sci. Soc. Am. J., 66, 1218-1224, 2002.

Lajtha, K., Bloomer, S. H.: Factors affecting phosphate sorption and phosphate retention in a desert ecosystem. Soil Sci., 146, 160–167, 1988.

Liu, L., Greaver, T. L.: A global perspective on belowground carbon dynamics under nitrogen enrichment. Ecol. Lett., 13, 819-828, 2010.

Liu, W., Zhang, Z., and Wan, S.: Predominant role of water in regulating soil and microbial respiration and their responses to climate change in a semiarid grassland. Global Change Biol., 15, 184-195, 2009.

Lombini, A., Llugany, M., Poschenrieder, C., Dinelli, E., and Barceló, J.: Influence of the Ca/Mg ratio on Cu resistance in three Silene armeria ecotypes adapted to calcareous soil or to different, Ni-or Cu-enriched, serpentine sites. J. Plant Physiol., 160, 1451-1456, 2003.

Lu, X., Mao, Q., Gilliam, F. S., Luo, Y., and Mo, J.: Nitrogen deposition contributes to soil acidification in tropical ecosystems. Global Change Biol., 20s, 3790-3801, 2014.

Lucas, R. W., Klaminder, J., Futter, M. N., Bishop, K. H., Egnell, G., Laudon, H., Högberg, P.: A meta-analysis of the effects of nitrogen additions on base cations: implications for plants, soils, and streams. Forest Ecol. Manag., 262, 95-104, 2011.

Lü, X., Reed, S., Yu, Q., He, N., Wang, Z., Han, X.: Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. Global Change Biol., 19, 2775-2784, 2013.

Lü, L., Wang, R., Liu, H., Yin, J., Xiao, J., Wang, Z., Zhao, Y., Yu, G., Han, X., and

Jiang, Y.: Effect of soil coarseness on soil base cations and available micronutrients in a semi-arid sandy grassland. Solid Earth, 7, 549-556, 2016.

Loew, O.: Uber die physiolgischen funkton der kalzium- und magnesia-salze in planzen organisms. Flora, 75, 368–394, 1892.

5 Lynch, J. P., and Clair, S. B. S.: Mineral stress: the missing link in understanding how global climate change will affect plants in real world soils. Field Crop. Res., 90, 101-115, 2004.

Malhi, S. S., Nyborg, M., and Harapiak, J. T.: Effects of long-term N fertilizer-induced acidification and liming on micronutrients in soil and in
10 bromegrass hay. Soil Till. Res., 48, 91-101, 1998.

Mikha, M. M., and Rice, C. W.: Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. Soil Sci. Soc. Am. J., 68, 809-816, 2004.

McLeod, S.: Micro-distillation unit for use in continuous flow analyzers. Its construction and use in determination of ammonia and nitrate in soils. Anal Chim.
15 Acta, 266, 107-112, 1992.

Menge, D. N. and Field CB.: Simulated global changes alter phosphorus demand in annual grassland. Global Change Biol., 13, 2582-2591, 2007.

Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27, 31-36, 1962.

20 Neff, J., Townsend, A. R., Gleixner, G., Lehman, S., Turnbull, J., and Bowman, W.: Variable effects of nitrogen additions on the stability and turnover of soil carbon. Nature, 419, 915-917, 2002.

Olsen, S. R., Watanabe, F. S., Cosper, H. R., Larson, W. E., and Nelson, L. B.: Residual phosphorus availability in long-term rotations on calcareous soils. Soil Sci., 78, 141-152, 1954.

Oorts, K., Vanlauwe, B., and Merckx, R.: Cation exchange capacities of soil organic matter fractions in a Ferric Lixisol with different organic matter inputs. Agri. Ecosyst. Environ., 100, 161-171, 2003.

Piccolo, A.: The supramolecular structure of humic substances. Soil science, 166(11), 810-832, 2001.

Reich, P. B., Tjoelker, M. G., Pregitzer, K. S., Wright, I.J., Oleksyn, J., Machado, J. L.: Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. Ecol. Lett., 11, 793-801, 2008.

Rogovska, N. P., Blackmer, A. M., and Mallarino, A. P.: Relationships between soybean yield, soil pH, and soil carbonate concentration. Soil Sci. Soc. Am. J., 71, 1251-1256, 2007.

Sardans, J. and Peñuelas, J.: The role of plants in the effects of Global Change on nutrient availability and stoichiometry in the plant-soil system. Plant Physiol., 160, 1741-1761, 2012.

Schmidt, E. L.: Nitrification in Soil. In: Stevenson FJ (Ed), Nitrogen in Agricultural Soils. Madison, WI, USA: American Society of Agronomy, 1982.

Six, J., Bossuyt, H., Degryze, S., Denef, K.: A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res., 79, 2004.

Six, J. and Paustian, K.: Aggregate-associated soil organic matter as an ecosystem

property and a measurement tool. Soil Biol. Biochem., 68, A4-A9, 2014.

Tian, Q., Liu, N., Bai, W., Li, L., and Zhang, W.: Disruption of metal ion homeostasis

in soils is associated with nitrogen deposition-induced species loss in an Inner

Mongolia steppe. Biogeosciences, 12, 3499-3512, 2015.

Tian, Q., Liu, N., Bai, W., Li, L., Chen, J., Reich, P. B., Yu Q., Guo, D. L., Smith, M.

D., Knapp, A. K., Cheng, W., Lu, P., Gao, Y., Yang, A., Wang, T., Li, X., Wang, Z.,

Ma, Y., Han, X., and Zhang, W.: A novel soil manganese mechanism drives plant

species loss with increased nitrogen deposition in a temperate steppe. Ecology, 97,

65-74, 2016.

Tunesi, S., Poggi, V., and Gessa, C.: Phosphate adsorption and precipitation in

calcareous soils: the role of calcium ions in solution and carbonate minerals. Nutri.

Cycl. Agroecosys., 53, 219–227, 1999.

Wang, R., Dungait, J. A., Creamer, C. A., Cai, J., Li, B., Xu, Z., Zhang, Y., Ma, Y., and

Jiang, Y.: Carbon and nitrogen dynamics in soil aggregates under long-term

nitrogen and water addition in a temperate steppe. Soil Sci. Soc. Am. J., 79,

527-535, 2015a.

Wang, R., Dorodnikov, M., Yang, S., Zhang, Y., Filley, T. R., Turco, R. F., Zhang, Y.,

Xu, Z., Li, H., and Jiang, Y.: Responses of enzymatic activities within soil

aggregates to 9-year nitrogen and water addition in a semi-arid grassland. Soil Biol.

Biochem., 81, 159-167, 2015b.

Wang, R., Creamer, C. A., Wang, X., He, P., Xu, Z., and Jiang, Y.: The effects of a

9-year nitrogen and water addition on soil aggregate phosphorus and sulfur availability in a semi-arid grassland. Ecol. Indic., 61, 806-814, 2016.

Wei, C., Yu, Q., Bai, E., Lü, X., Li, Q., Xia, J., Kardol, P., Liang, W., Wang, Z., and Han, X.: Nitrogen deposition weakens plant–microbe interactions in grassland ecosystems. Global Change Biol., 19, 3688-3697, 2013.

Wiesmeier, M., Steffens, M., Mueller, C. W., Köbl, A., Reszkowska, A., Peth, S., Horn, R., and Kögel-Knabner, I.: Aggregate stability and physical protection of soil organic carbon in semi-arid steppe soils. Eur. J. Soil Sci., 63, 22-31, 2012.

Wright, I. J.: Phosphorus sequestration in soil aggregates after long-term tillage and cropping. Soil Till. Res., 103, 406-411, 2009.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., Diemer, M., et al.: The worldwide leaf economics spectrum. Nature, 428, 821-827, 2004.

Wu, H., Wiesmeier, M., Yu, Q., Steffens, M., Han, X., and Kögel-Knabner, I.: Labile organic C and N mineralization of soil aggregate size classes in semiarid grasslands as affected by grazing management. Biol. Fertil. Soils, 48, 305-313, 2012.

Xu, Z., Wan, S., Ren, H., Han, X., Li, M. H., Cheng, W., and Jiang, Y.: Effects of water and nitrogen addition on species turnover in temperate grasslands in northern China. PloS ONE, 7, e39762, 2012.

Young, I. M. and Ritz, K.: Tillage, habitat space and function of soil microbes. Soil Till. Res., 53: 201-213, 2000.

Table 1 The concentrations of dissolved organic carbon (DOC, mg kg soil aggregate[-1]), dissolved inorganic nitrogen (DIN, mg kg soil aggregate[-1]), available phosphorus (AP, mg kg soil aggregate[-1]), exchangeable Ca and Mg (cmol kg soil aggregate[-1]), and available Fe and Mn (mg kg soil aggregate[-1]) within bulk soil and soil fractions of 0-10 cm soils without field-manipulated treatments.

	Bulk soil	Soil aggregates (μm)		
		> 2000	250-2000	< 250
DOC	69.8±5.6	72.5±6.8	66.6±1.3	71.2±1.5
DIN	21.6±1.5	21.1±0.6	49.5±1.9	57.3±2.0
AP	14.9±2.8	3.7±0.4	5.8±0.7	3.9±0.5
Ca	19.6±0.3	26.6±0.7	19.8±0.4	22.7±2.7
Mg	1.7±0.05	1.8±0.1	1.4±0.05	2.0±0.1
Fe	21.8±0.6	24.0±1.8	20.0±1.4	27.5±2.8
Mn	23.3±2.7	24.5±0.8	20.0±0.8	24.0±1.9

Table 2 Two-way ANOVAs (*F* values) on the effect of soil aggregate size (S),

nitrogen addition (N), and their interactions on the ratios of DOC:DIN, DOC:AP,

DIN:AP, exchangeable Ca:Mg, and available Fe:Mn.

	DOC:DIN	DOC:AP	DIN:AP	Ca:Mg	Fe:Mn
S	100.86^{**}	0.41	16.32^{**}	1.81	0.25
N	36.91^{**}	3.44^{*}	1.73	1.19	109.23^{**}
N×S	12.64^{**}	1.72	2.43*	2.07	7.41^{**}

5 * Significance level at $P < 0.05$; ** Significance level at $P < 0.01$.
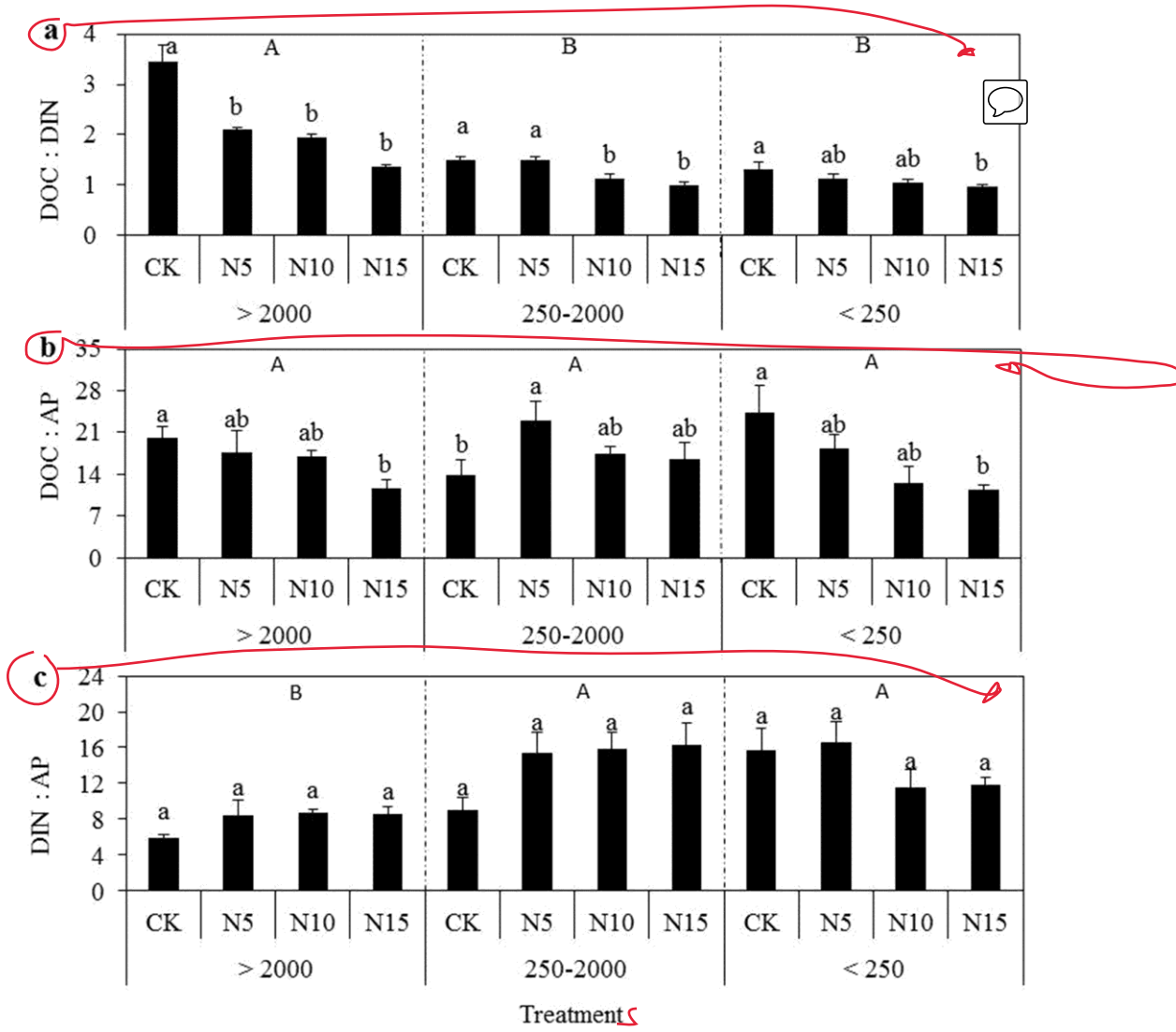
Fig. 1 The ratio of (a) dissolved organic carbon to dissolved inorganic nitrogen (DOC:DIN), (b) DOC to available phosphorus (DOC:AP), and (c) DIN:AP as influenced by N addition in soil aggregates. Data are represented as mean±SE. Lowercase letters indicate significant differences between N treatments within a soil fraction. The capital letters at the top indicate significant differences between soil aggregate sizes across N treatments.
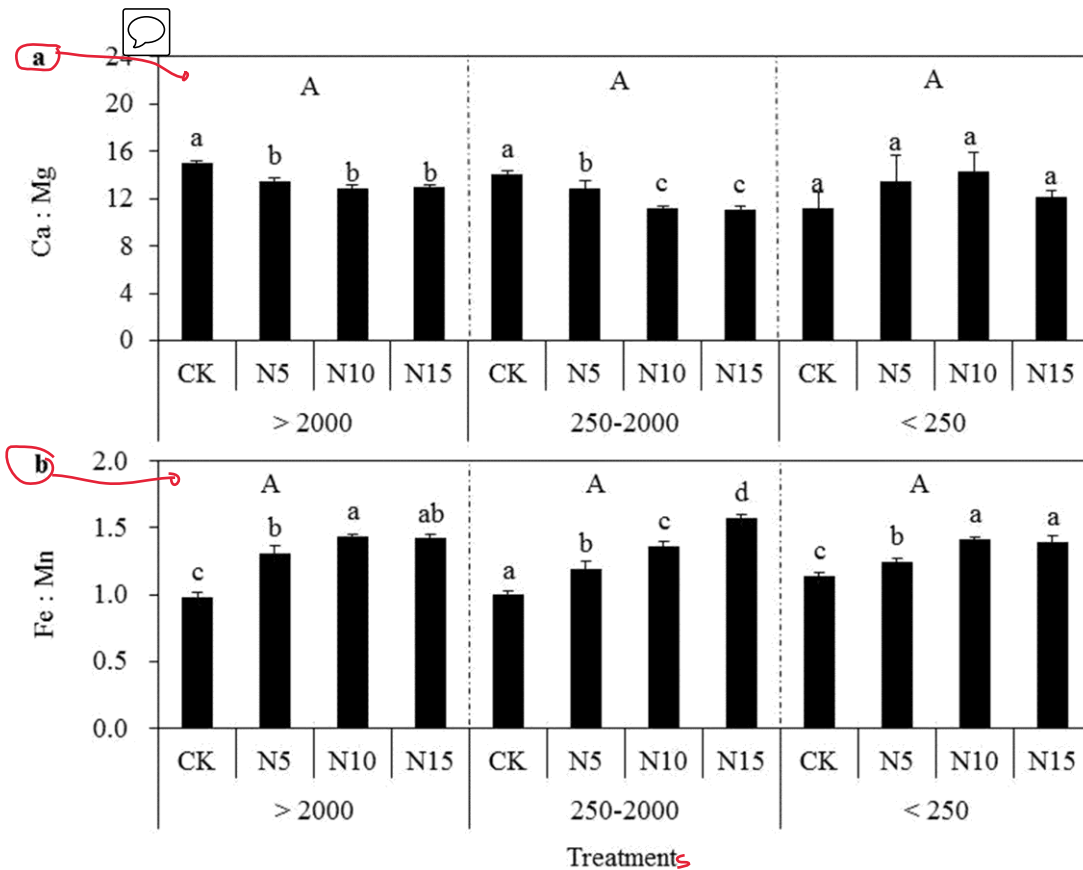
Fig. 2 The ratio of (a) exchangeable Ca:Mg and (b) available Fe:Mn as influenced by

N addition in soil aggregates. Data are represented as mean±SE. Lowercase letters

indicate significant differences between N treatments within a soil fraction. The

5   capital letters at the top indicate significant differences between soil aggregate sizes

across N treatments.
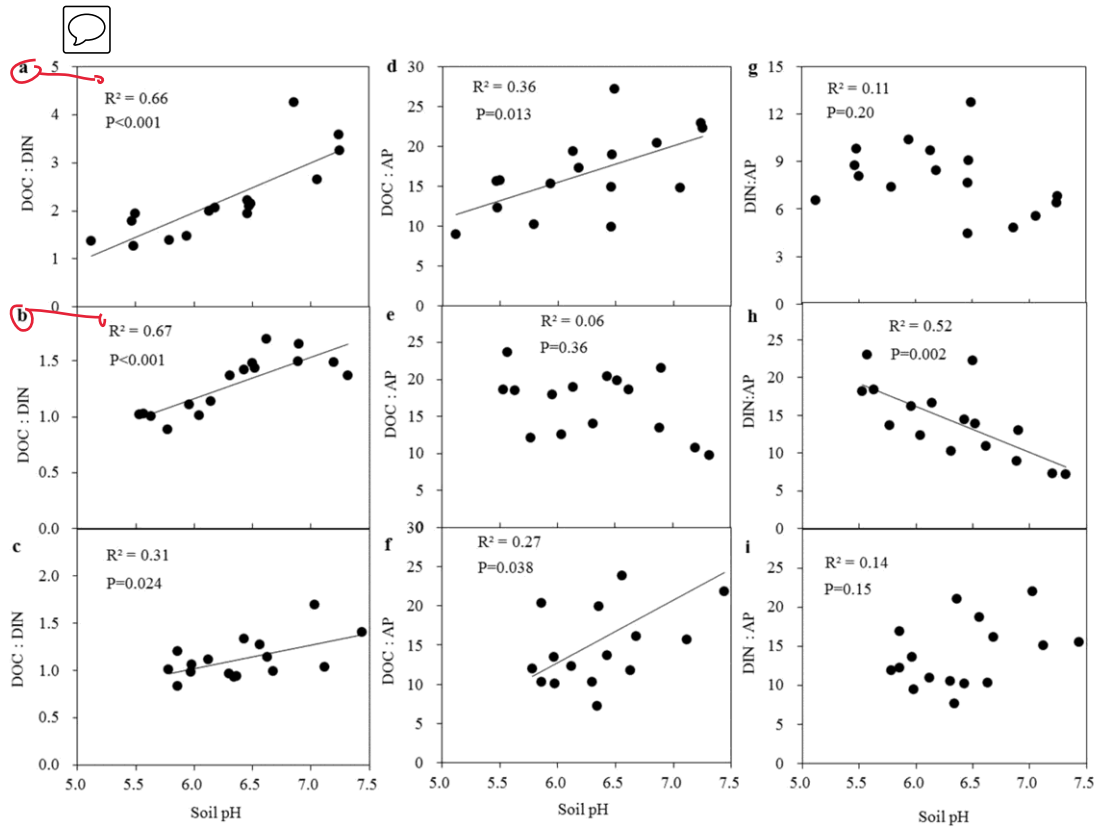
Fig. 3 The relationships between soil pH and DOC:DIN within large macroaggregates

(a), small macroaggregates (b), and microaggregates (c), between soil pH and

DOC:AP within large macroaggregates (d), small macroaggregates (e), and

microaggregates (f), and between soil pH and DIN:AP ratio within large

macroaggregates (g), small macroaggregates (h), and microaggregates (i).

Significance level was set at $P < 0.05$.
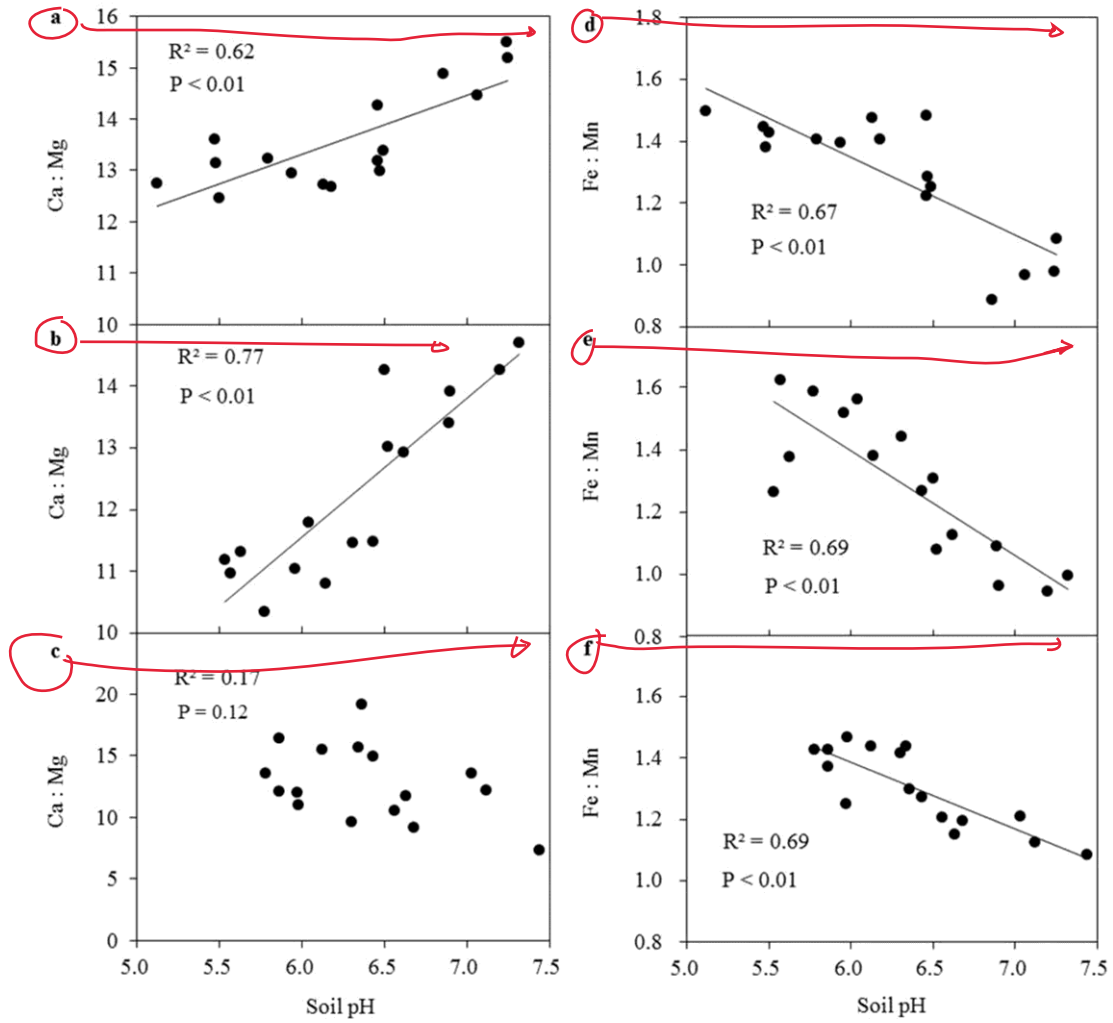
Fig. 4 The relationships between soil pH and exchangeable Ca:Mg within large

macroaggregates (a), small macroaggregates (b), and microaggregates (c), and

between soil pH and available Fe:Mn ratio within large macroaggregates (d), small

5   macroaggregates (e), and microaggregates (f). Significance level was set at P < 0.05.