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Base cations and micronutrients in soil aggregates as affected by enhanced nitrogen and water inputs in a semi-arid steppe grassland

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Abstract

The intensification of grassland management by nitrogen (N) fertilization and irrigation may threaten the future integrity of fragile semi-arid steppe ecosystems by affecting the concentrations of base cation and micronutrient in soils. We extracted base cations ~~(~~ of exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) and extractable micronutrients of iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) from three soil aggregate sizes classes (microaggregates, <0.25 mm; small macroaggregates, 0.25-2 mm; large macroaggregates, >2 mm) from a 9-year N and water field manipulation study. There were significantly more base cations (but not micronutrients) in microaggregates compared to macroaggregates which was related to greater soil organic matter and clay contents. Nitrogen addition significantly decreased exchangeable Ca by up to 33% in large and small macroaggregates and exchangeable Mg by up to 27% in three aggregates but significantly increased extractable Fe, Mn and Cu concentrations (by up to 262%, 150%, and 55 %, respectively) in all aggregate size classes. However, water addition only increased exchangeable Na, while available Fe and Mn were decreased by water addition when averaging across all N treatments and aggregate classes. The loss of exchangeable Ca and Mg under N addition and extractable Fe and Mn in soil aggregates under water addition might potentially constrain the productivity of this semi-arid grassland ecosystem.

Key words Nitrogen deposition • Irrigation • Calcium • Magnesium • Manganese •

Copper

Introduction

Micronutrient availability constrains net primary productivity (NPP) (Li et al., 2010, 2012), and deficiencies in soil micronutrients, including Fe, Mn, Cu, and Zn, are a problem threatening food production worldwide (Jones et al., 2013). Base cations (i.e., exchangeable Ca, Mg, K, Na) are the predominant exchangeable cations in the calcareous soils (Zhang et al., 2013). They are essential for soil buffering capacity particularly in soils affected by atmospheric acid deposition (Lieb et al., 2011), serve as good indicators of soil fertility (Zhang et al., 2013), and are critical nutrients for both plant and microbial metabolism (Cheng et al., 2010). For instance, Ca regulates plant cell signaling, cell division, and carbohydrate metabolism (McLaughlin and Wimmer, 1999), and Mg is important for photosynthesis and energy storage (Lucas et al., 2011). Biogeochemical processes may be driven by base cation and micronutrient supply; for instance, root-surface phosphatase activity is correlated with available Ca and Mg (Gabbrielli et al., 1989) and Mg and Zn availability are important for litter decomposition (Powers and Salute, 2011).

The availability of base cations and micronutrients is influenced by environmental changes, such as altered N and water ~~availabilities~~ availability (Treseder, 2008). The availability of base cations varies with edaphic properties, such as soil pH (Katou, 2002), organic matter fractions (Oorts et al., 2003) and soil particle sizes (Beldin et al., 2007). Prolonged N inputs generally causes soil acidification and subsequent losses of soil cations (McLaughlin and Wimmer, 1999; Cheng et al., 2010), and micronutrient availability may increase under soil acidification (Malhi et al., 1998) causing toxicity to both plants and soil microorganisms in extreme cases (Bowman et al., 2008; Horswill et al., 2008). Changes in precipitation regime and soil moisture levels may interact with inorganic N affecting soil microbial activities (Wang et al., ~~2014,~~ 2015a) including the decomposition of soil organic matter (SOM) and nutrients release and their subsequent transport in the soil (Dungait et al., 2012; Nielsen and Ball, 2014). In sandy soils, increased precipitation might promote leaching of nitrate and counter-ions (such as base cations) (Ochoa-Hueso et al., 2014).

Soil aggregate structure predominantly controls SOM dynamics (Six et al., 2004) and microbial activities (Dorodnikov et al., 2009), and soil aggregate stability can serve as an indicator for grassland ecosystem health (Reinhart et al., 2015). In comparison with macroaggregates, microaggregates provide preferential sites for soil C stabilization (Wang et al., 2015b) and the SOM herein is more microbial-processed as evidenced by natural abundance stable ^{13}C values (Gunina and Kuzyakov, 2014; Wang et al., 2015b). More microbial-processed SOM (i.e. more functional groups) and potentially higher mineral contents within microaggregates (Creamer et al., 2011) would purportedly provide more binding sites for base cations and micronutrients. Therefore, dynamics of soil base cations and micronutrients in aggregate scale would be a good indicator for soil health and for the potential of metal nutrients sustainability. However, studies concerning aggregate-scale distribution of base cations and micronutrients under enhanced N input and precipitation are still rarely seen.

Semi-arid steppe grasslands support diverse animal and plant species (Kang et al., 2007) and are experiencing or will experience enhanced atmospheric nitrogen (N) deposition and precipitation (Niu et al., 2009; Bai et al., 2010;~~ Wang et al., 2014, 2015a;~~ Zhang et al., 2014). Previously, we demonstrated that water addition promoted the incorporation of microaggregates into macroaggregates and enhanced decomposition rates within microaggregates compared to macroaggregates, and that the addition of N depressed extracellular enzyme activities within soil aggregates as a result of soil acidification, after 9-years in a field experiment in the semi-arid steppe grasslands of Inner Mongolia (Wang et al., 2015a,b). In this study, we investigated the changes of base cations and micronutrients within the soil aggregates. We hypothesized that (1) both concentrations of base cations and micronutrients would increase in microaggregates because of the increased abundance of adsorption sites provided by greater SOM and mineral contents therein, and (2) that increased N and water inputs would decrease base cations and increase the availability of micronutrients within soil aggregates due to soil acidification and leaching in the free-draining soil.

Materials and methods

Study sites and experiment design

The experiment was conducted in the Inner Mongolia Restoration Ecological Research Station (IMRERS) in the south of Duolun County, Inner Mongolia, China (42°02′27″N, 116°17′59″E, elevation 1,324 m a.s.l). The topography of the experimental area is flat. The mean annual temperature is 2.1 ºC, ranging from -17.8 ºC in January to 18.8 ºC in July, and mean annual precipitation is 379.4 mm (Xu et al., 2012). The soil is a Haplic Calcisols according to the FAO classification (IUSS Working Group WRB, 2015) with a texture of sandy loam (0-10 cm): 63% sand, 20% silt, and 17% clay (Wang et al., 2014). The chemical characteristics of the 0 – 10 cm depth of whole soil are given in Wang et al. (2014).

The experimental design is described in detail in Wang et al. (2014). Briefly, experimental plots were set up and run for 9 years. A split-plot design was applied with water and N addition being the two treatments. In April 2005, twelve 8 m × 8m plots were established in each of seven treatment blocks with 1 m buffer zone between any two adjacent plots. Each block was divided into two main plots with ~~Based on water treatment (~~either ambient precipitation ~~and~~ or 180 mm water addition)~~, each of seven blocks was divided into two main plots~~ as treatments. Each main plot was divided into six subplots and nitrogen treatments (urea pellets) were applied to a randomly selected subplot (dispersed on the top of the soil) (Xu et al., 2012). Two subplots were phosphorus addition treatments which were not considered in this study. Additional water (approximately 50% of mean annual precipitation) was added to the water addition plots by sprinkling 15 mm weekly during the middle of the growing season from June to August as over 65% of annually total precipitation occurs during this time. The chemical composition of the irrigation water was listed as Table S1. Nitrogen additions were split applications with half applied in early May and the other half in late June at the rates of 0 (control plots, defined as CK), 5 (N_5), 10 (N_{10}), and

15 (N_{15}) g N m^{-2} yr^{-1} (Xu et al., 2012). The background N inputs (atmospheric deposition plus fertilizer application) in this area are about 5 g N m^{-2} yr^{-1}, so these manipulations represent 100%, 200% and 300% surplus of nitrogen compared to the background N inputs (Wang et al., 2015a).

Soil sampling and aggregate-size fractionation

In September 2013, top soils (0 – 10 cm) were sampled by compositing five randomly selected cores within each plot from four out of seven blocks. Fresh soil samples were stored at 4ºC during transportation to the laboratory. Soil aggregates were isolated by a dry-sieving method according to Dorodnikov et al. (2009) to minimize the disruption in microbial activities and to prevent leaching of available nutrients by wet sieving. Briefly, fresh bulk soil samples (gravimetric water content of 10-15%) were gently passed through a 5 mm screen and transferred to a nest of sieves (2 and 0.25 mm) on a mechanical shaker Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany). The sieves were mechanically shaken (amplitude 1.5 mm) for 2 min to separate the aggregates > 2 mm (large macroaggregates), 2 – 0.25 mm (small macroaggregates), and < 0.25 mm (microaggregates). The mass proportions of large macro-, small macro-, and microaggregates were 30.3-44.8%, 38.3-42.8%, and 15.3-31.1%, respectively (Wang et al., 2015b). No correction was made for sand because sand grains can be completely embedded within macroaggregates. The chemical characteristics of bulk soil and soil aggregates (0-10 cm) are given in Table 1 (Wang et al., 2014, 2015a,b).

Soil physiochemical and biological parameters

Soil aggregate pH and electrical conductivity (EC) was determined in a 1:5 (w/v) soil-to-water extract with a PHS-3G digital pH meter (Precision and Scientific Corp., Shanghai, China) and a Orion 150A+ conductivity meter (Thermo Fisher Scientific Inc., Beverly, USA), respectively. Soil organic carbon (SOC) within aggregate classes was determined for acid-fumigated soils (to remove carbonates) using elemental analyzer (EA1112, ThermoFisher Scientific, Japan). Concentration of soil aggregate ammonium was determined colorimetrically from 2 M KCl soil extracts (Wang et al., 2015b).

Extraction and analysis of base cations and micronutrients

Soil aggregate base cations were extracted and analysed using the method of Ochoa-Hueso et al. (2014). Briefly, 2.5 g of each soil aggregate fraction was extracted by 50 ml 1 M ammonium acetate (CH_3COONH_4) (pH 7.0). The slurry was orbitally shaken for 30 min at 150 rpm and then filtered through Whatman no. 2V filter paper (quantitative and ash-free). Micronutrients (Fe, Mn, Cu, Zn) within soil aggregates were extracted by diethylenetriamine pentaacetic acid (DTPA) (Lindsay and Norvell, 1978). 10 g of soil aggregates was weighted into Erlenmeyer flask to mix with 20 mL 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA (triethanolamine) (pH 7.0). The mixture were shaken at 180 rpm for 2 h and filtered. All of the extractable metal cations were analyzed by atomic absorption spectrometer (AAS, Shimadzu, Japan). The sum of exchangeable Ca, Mg, K, and Na was defined as effective cation exchange capacity (ECEC).

Statistical analyses

~~A split-plot design~~ Three-way ANOVAs with a split-plot design ~~was~~ were used to test the effect of water (between subject), N addition (within subject) and soil aggregate sizes on electrical conductivity (EC), the concentrations of base cations (exchangeable Ca, Mg, K, and Na) and available micronutrients (Fe, Mn, Cu, and Zn). ~~The effects of N addition rates on~~Multiple comparisons with Duncan design were performed to determine differences in EC, base cations, and available micronutrients ~~were determined by one-way ANOVA~~among N rates and run separately for ambient and enhanced precipitation within each soil aggregate. Pearson correlation analysis was used to examine the relationships between base cations and available micronutrients. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, U.S.A.) and statistical significance was accepted at $P<0.05$.

Results

Responses of electrical conductivity (EC) ~~and total base cations~~ to N and water addition

Under ambient water conditions, soil EC was significantly reduced in N addition plots from 272.5 to 179.2 µs cm^{-1} in large macroaggregates, from 249.4 to 188.6 µs cm^{-1} in small macroaggregates, and from 295.3 to 173.9 µs cm^{-1} in microaggregates~~in soil~~

~~aggregate size classes~~, except for N_5 in large macro- and microaggregates as compared to the control (CK) (Fig. 1a, Table 2). Under elevated water conditions, EC values were significantly smaller in N_{15} plots across soil aggregate size classes (Fig. 1a). The N_{15} treatment significantly decreased soil EC from 237.8 to 194.5 µs cm^{-1} in large macroaggregates, from 247.5 to 192.9 µs cm^{-1} in small macroaggregates, and from 293.5 to 192.7µs cm^{-1} in microaggregates (Fig. 1a). Soil EC values were significantly larger in water addition plots at N application rates of N_{10} in large macroaggregates, N_5 and N_{10} in small macroaggregates, and N_{10} in microaggregates comparing to respective N plots of ambient precipitation (Fig. 1a).

~~Total base cations (sum of exchangeable Ca, Mg, K, and Na) in soil aggregates increased in the order: small macroaggregates < large macroaggregates < microaggregates averaging across all N and water treatments (Table 2, Fig. 1b). Soil base cations decreased with N addition in both large and small macroaggregates under both ambient and elevated water conditions (Fig. 1b).~~

Response of ECEC, ~~Exchangeable~~ exchangeable Ca, Mg, K, and Na and DTPA-extractable micronutrients to N and water addition

T~~otal base cations~~he ECEC (sum of exchangeable Ca, Mg, K, and Na) in soil aggregates increased in the order: small macroaggregates < large macroaggregates < microaggregates averaging across all N and water treatments (Table 2, Fig. 1b). Soil base cations decreased with N addition in both large and small macroaggregates under both ambient and elevated water conditions (Fig. 1b).

When averaging all the N and water treatments, the greatest concentrations of exchangeable Ca, Mg, and K were all detected in microaggregates, followed by large macroaggregates (Fig. 2a, b, c). Microaggregates contained significantly more exchangeable Na than the large macroaggregates averaging across all the treatments (Fig. 2d). Under ambient precipitation, exchangeable Ca was significantly less in N-addition plots with N_5, N_{10}, and N_{15} in large macroaggregates, and in N_{10} and N_{15} plots in small macroaggregates as compared to control plots by as much as 33 % (between CK and N_{15} treatments) (Fig. 2a). Under elevated water conditions, N addition significantly decreased exchangeable Ca in both large (N_5, N_{10}, and N_{15}) and small macroaggregates (N_{10} and N_{15}) by as much as 26 % (Fig. 2a). Under ambient water conditions, exchangeable Mg was significantly lower in N_{15} in large macroaggregates, in N_5 and N_{15} in small macroaggregates, and in N_{15} in microaggregates by 4 - 27 % (Fig. 2b). Under increased water inputs, N addition decreased exchangeable Mg in N_{10} and N_{15} plots in both large and small macroaggregates and in N_5, N_{10}, and N_{15} in microaggregates by 2 - 19 % (Fig. 2b). Both exchangeable K and Na showed no response to N addition across all aggregate-size classes (Fig. 2c, d). Significant positive water effects were only seen on the concentration of exchangeable Na when comparing the means of all N treatments and aggregate classes between ambient precipitation and water addition (Table 2).

DTPA-extractable micronutrients were distributed differently across aggregate-size classes (Fig. 3, Table 2). Significantly less extractable Fe and Mn were detected within small macroaggregates compared to large macro- and microaggregates (Fig. 3a, b). Extractable Cu increased in the order large macro- > micro- > small macroaggregates when averaging all the N and water treatments (Fig. 3c). The largest extractable Zn was observed in microaggregates, while no significant difference was found for Zn between large and small macroaggregates (Fig. 3d). Following N additions, DTPA-extractable Fe, Mn, and Cu significantly increased within all soil aggregates under both ambient and elevated water conditions by as much as 262 %, 150 %, and 55 %, respectively (Fig. 3a, b, c). No overall N effects were detectable on extractable Zn (Table 2), but Zn decreased significantly with N_{10} and N_{15} treatments under water addition within microaggregates (Fig. 3d). Extractable Fe and Mn significantly decreased with water addition under N addition rates of 5, 10, and 15 g N m^{-2} yr^{-1} within large macroaggregates (Fig. 3a,b, Table 2).

Correlations between soil physicochemical parameters, base cations and micronutrients

Significant positive correlations were observed between SOC and soil exchangeable Ca, Mg, and K, and between pH and EC, exchangeable Ca, Mg, and Na (Table 3). There were significant positive relationships between exchangeable Ca vs. Mg, Ca vs. K, Ca vs. K, Mg vs. K, and Mg vs. Na (Table 3). Micronutrients Fe, Mn, and Cu were negatively correlated with soil pH across all soil aggregate size classes (Fig. 4a, b, c). Within macroaggregates, ~~total base cations~~ECEC significantly and negatively correlated with extractable ammonium (~~Table ~~Fig. ~~S1a~~5a).

Discussion

Distribution of base cations and micronutrients within soil aggregates

Relatively increased clay mineral and SOM contents are commonly recognized to be associated with the smallest aggregate size fractions (< 0.25 mm), both of which play an essential role in retaining soil base cations especially in sandy, free-draining soils similar to our experimental field plot (Oorts et al., 2003; Beldin et al., 2007). In line with our initial hypothesis, a significant positive correlation was detected between SOC (the largest component of SOM) and exchangeable Ca, Mg, K, and ~~total base cations~~ECEC (Table 3), and their distribution was similar to the distribution pattern of SOC and total nitrogen with the highest concentrations in microaggregates (Wang et al., 2015b). Soil organic matter was more decomposed within microaggregates (Wang et al., 2015b) and thus may have provided more binding sites (e.g. more carbonyl functions; Golchin et al., 1994) for exchangeable base cations. It is widely reported that available micronutrients correspond to both SOM and clay content which are both usually more abundant in microaggregates (e.g. Sharma et al., 2004; Six et al., 2004). Although we observed greater available Zn in microaggregates compared to macroaggregates ~~(as reported by Głąb and Gondek, 2014)~~, there was no significant relationship of SOC with Fe, Mn, and Cu, which had quite different distribution patterns from SOC within soil aggregate-size classes and was similar to the findings by Głąb and Gondek (2014) in bulk soil. Due to the fact that large macroaggregates are responsible for providing nutrients for plants and the early stages of SOM formation (Six et al., 2004), plant cycling (uptake and biomass decomposition) might contribute to the higher concentrations of Fe, Mn and Cu in large macroaggregates (Jobbágy and Jackson, 2001).

Response of EC and base cations to nitrogen and water addition

Protons released when soils acidify may exchange with base cations making them available for take-up by plants or transport (and loss) by water in soils (Lucas et al., 2011; Malhi et al., 1998; Sharma et al., 2004). As EC represents salt concentration in soil solution (Zhou et al., 2011), significantly lower EC values might suggest the loss of ions, in line with the decline of ECEC under higher N rates (Fig. 1a vs. Fig. 1b). Deficiencies in soil base cations caused by N deposition could pose a threat to ecosystems through their effect on soil microbial activity (Vitousek et al., 1997; Treseder, 2008). Many studies on the effects of chronic N addition to soil contribute shifts in soil microbial community structure and activity to the reduction of soil pH (Ramirez et al., 2010; Wang et al., 2014), and we previously reported soil enzyme depression under N addition attributed to soil acidification (Chung et al., 2007; Wang et al., 2014). Consistent with our hypothesis, we observed a decrease in Ca and Mg, which are the major base cations in macroaggregates (Fig. 2). This decrease corresponded to a negative correlation between NH_4^+ and ~~total base cation concentrations~~ECEC across soil macroaggregates (Fig. ~~S1~~5a). Nitrogen addition increased NH_4^+ concentrations across all three soil aggregate size classes (Wang et al., 2015b) which may displace soil aggregate base cations and bind strongly to soil surfaces (Matschonat and Matzner, 1996). We did not measure leaching rates in the field experiment, but these are likely to be substantial given the sand content of the soil (> 60%). Increased leaching under water addition that caused NO_3^- loss was previously reported (Wang et al., 2015b) and may indicate a route for the loss of Ca and Mg within soil aggregates, as observed by Currie et al. (1999) and Lucas et al. (2011). Our results contradict those of Ochoa-Hueso et al. (2014) who reported an increase of extractable base cations under N deposition in a highly buffered, limestone soil in the semi-arid Mediterranean region. However, where N addition increases NPP in an N-limited soil, such as those of semi-arid steppe grasslands (Xu et al., 2014) a coincident uptake of base cations by vigorous plant growth may occur (Tomlinson, 2003). But we did not investigate changes in the cation content of plant tissues in this experiment to confirm this flux. Furthermore, no effect of N addition was observed on soil aggregate K and Na concentrations (although Ca and Mg decreased; Fig. ~~3~~2, Table 2) indicating the selective depletion of base cations (Lu et al., 2014). The depletion of base cations within soil aggregate can accelerate a reduction in the buffering capacity of soils and accelerate soil acidification (with increased N at ambient water soil pH 6.97 decreased to 6.10, and with added water, 7.17 to 6.42; Wang et al., 2015a); the dynamics of the base cations were affected substantially. Water addition increased EC and exchangeable Na but did not affect the other base cations (Table 2; Fig. 1a, 2). ~~Although the Na concentration in the water for irrigation is uncertain, t~~The positive effect of water on soil EC and exchangeable Na ~~was unlikely~~might be caused by ~~Na~~ the relatively high EC value and Na concentration in irrigative water ~~due to the undetectable water effect on Ca~~ in this calcareous grassland (Table S1). We previously reported that water addition increased soil microbial activity at the study site (Wang et al., 2014, 2015a): if litter decomposition rates were promoted as a consequence (e.g. Liu et al., 2006) Ca, Mg and K may have been released from this secondary biological source to supplement the soil pools.

Response of micronutrients to nitrogen and water addition

Micronutrient cycling relies on the balance between input (mineral weathering release), recycling (plant biomass turnover), retention (sorption reactions), and removal (biomass harvesting, fire, and hydrologic leaching) (Li et al., 2008). Long-term N addition caused large increases in extractable Fe, Mn and Cu concentrations in all soil aggregate size classes (by up to 262%, 150%, and 55 %, respectively; Fig. 3a, b, c), presumably due to the decrease in soil pH which may increase micronutrient release through weathering and desorption from soil minerals and SOM (Malhi et al., 1998). Acidification caused by the long-term addition of N negatively affected microbial activity previously observed as the depression of enzyme activity in these experimental soils (Chung et al., 2007; Wang et al., 2014). The mechanism underlying this biological response was not explored but is likely to be complex and due in part to the effect of the changes in micronutrient supply. Long-term irrigation had the opposite effect on extractable Fe and Mn within soil aggregates where increases in soil aggregate pH (which usually decrease the mobilization of micronutrients) were detected compared to ambient precipitation plots

(Wang et al., 2014). Our previous study suggested that water addition increased soil pH from 7.1 to 7.3 in CK and from 5.6 to 6.1 in N_{15} plots for large macroaggregates, from 7.1 to 7.5 in CK and from 5.8 to 6.2 in N_{15} plots for small macroaggregates, and from 7.1 to 7.4 in CK and from 5.9 to 6.2 in N_{15} plots for microaggregates (Wang et al., 2015). A previous study carried out at the same plots showed that water addition significantly increased NPP (Xu et al., 2010), suggesting enhanced uptake of growing plants may have reduced concentrations of Fe and Mn in the soil aggregates. This is consistent with the findings of Li et al. (2008) who reported that Mn and boron uptake by plants outpaced their resupply by mineral weathering. However, Fe and Mn may be toxic to plants (Brown and Jones, 1977), so losses by increased leaching may be the most likely route for their decrease in the irrigated plots. However, neither leaching nor plant performance was measured in this experiment.

Conclusions

Consistent with our first hypothesis, microaggregates had greater base cation concentrations, which was ascribed to a proportion of sorption provided by increased SOM and clay contents associated with the smallest aggregate size class. However, extractable micronutrients (except Zn) did not follow the same concentration pattern as base cations across three soil aggregate size classes. Under N addition, exchangeable Ca and Mg decreased while extractable micronutrients (Fe, Mn and Cu) increased, which was related to the slight but apparently important decrease in soil pH. Water addition showed no impact on exchangeable Ca, Mg and K, but decreased available Fe and Mn averaging across soil aggregates which partially supported our second hypothesis. Our findings suggest that uncontrolled or long-term N fertilization and irrigation of semi-arid steppe soils cause significant changes in the concentrations of base cations and micronutrients which will ultimately decrease soil fertility, constrain NPP and the nutrient quality of plants. The pathway of the losses (e.g. leaching or uptake into plant tissues followed by harvest) need to be determined in order to manage large and fragile semi-arid grassland systems.

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Tables

Table 1 The chemical characteristics of bulk soil and soil aggregates without field-manipulated treatments. SOC, TN, C/N, and DOC represent soil organic carbon, total nitrogen, the ratio of SOC to TN, and dissolved organic carbon, respectively. Data are represented as means ± standard error.

		Soil aggregates (mm)		
	Bulk soil	> 2	0.25-2	< 0.25
SOC (g kg soil^{-1})	18.9±1.7	18.2 ± 1.0	15.1 ± 2.0	21.6 ± 1.4
TN (g kg soil^{-1})	1.8±0.2	1.9 ± 0.1	1.5 ± 0.2	2.2 ± 0.1
C/N	10.4±1.3	9.5 ± 0.5	10.4 ± 0.4	10.0 ± 0.2
pH	7.0±0.2	7.1 ± 0.2	7.1 ± 0.2	7.1 ± 0.3
DOC	69.8±5.6	72.5±6.8	66.6±1.3	71.2±1.5
NO_3^--N (mg kg soil^{-1})	27.3±5.9	4.2 ± 0.1	31.6 ± 2.2	34.4 ± 3.8
NH_4^+-N (mg kg soil^{-1})	9.4±1.6	17.0 ± 1.3	17.9 ± 2.5	22.9 ± 1.0

Data from Wang et al. (2014, 2015a,b)

Table 2 Results (*F* values) of ANOVAs with a split-plot design on the effects of N, water addition (W), and soil fractions (S) on soil electrical conductivity (EC), exchangeable Ca, Mg, K, Na, ~~total base cations~~effective cation exchange capacity (ECEC, sum of exchangeable Ca, Mg, K, and Na , cmol kg aggregate^{-1}), and available Fe, Mn, Cu, and Zn (mg kg aggregate^{-1}).

	EC	Ca	Mg	K	Na	~~Base cations~~ECEC	Fe	Mn	Cu	Zn
W	11.60*	3.07	5.08	0.17	15.6**	4.03	27.8**	14.1*	2.40	3.92
N	14.20**	6.36**	38.7**	0.33	1.71	7.36**	147**	136.3**	44.7**	2.47
S	3.63*	25.0**	168.3**	123.7**	3.49*	30.7**	17.7**	30.1**	33.7**	33.8**
W×N	6.90**	1.01	5.68**	6.81**	1.26	1.25	2.36	3.40*	7.23**	4.34**
W×S	0.28	1.03	3.75*	0.98	0.34	1.03	6.07**	10.8**	6.60**	2.32
N×S	0.77	0.87	0.36	0.88	1.81	0.88	1.59	2.43*	0.85	2.04
W×N×S	0.86	0.44	1.03	1.41	0.61	0.42	0.99	2.54*	1.16	3.84**

* and ** indicating the significant level at $P<0.05$, 0.01, respectively

Table 3 Correlation analyses (*R* values) among exchangeable base cations, effective cation exchange capacity (ECEC), soil organic carbon (SOC), soil pH, and soil electrical conductivity (EC).

	pH	EC	Ca	Mg	K	Na	~~Base cations~~ECEC
SOC	0.11	0.19	0.42**	0.73**	0.81**	0.17	0.46**
pH		0.66**	0.35**	0.48**	0.04	0.33**	0.37**
EC			0.28**	0.40**	0.15	0.16	0.29**
Ca				0.60**	0.53**	0.23*	0.998**
Mg					0.77**	0.24*	0.65**
K						0.10	0.57**
Na							0.26*

* and ** indicating the significant level at P<0.05, 0.01, respectively

Table S1 Chemical characteristics of the irrigation water.

	Irrigation water
EC (us cm^{-1})	196.1±6.4
pH	7.7±0.07
Ca (mmol L^{-1})	1.6±0.03
Mg (mmol L^{-1})	0.8±0.02
K (mmol L^{-1})	0.04±0.002
Na (mmol L^{-1})	0.6±0.02

The symbol ‘-’indicate the ions were not detected.

Figure Legends

Fig.1 The effect of 9-year of water (ambient vs. water addition) and N additions (0 [CK], 5 [N_5], 10 [N_{10}], 15 [N_{15}] g N m^{-2} yr^{-1}) on (a) electrical conductivity (EC, us cm^{-1}) and (b) ~~total base cations~~ effective cation exchange capacity (ECEC, sum of exchangeable Ca, Mg, K, and Na, cmol kg $aggregate^{-1}$). Data are represented as means ± standard error (n=4). Lowercase letters indicate significant differences between N treatments within a soil aggregate class size and water treatment. The capital letters indicate significant differences between soil aggregate sizes across N and water treatments.

Fig.2 The effect of 9-year of water (ambient vs. water addition) and N additions (0 [CK], 5 [N_5], 10 [N_{10}], 15 [N_{15}] g N m^{-2} yr^{-1}) on exchangeable (a) Ca, (b) Mg, (c) K and (d) Na concentrations (cmol kg $aggregate^{-1}$) in 3 soil aggregate size classes (<0.25 mm, 0.25-2 mm, >2 mm). Data are represented as means ± standard error (n=4). Lowercase letters indicate significant differences between N treatments within a soil aggregate size class and water treatment. The capital letters indicate significant differences between soil aggregate sizes across N and water treatments.

Fig. 3 The effect of 9-years of water (ambient vs. water addition) and N additions (0 [CK], 5 [N_5], 10 [N_{10}], 15 [N_{15}] g N m^{-2} yr^{-1}) on DTPA-extractable micronutrients (a) Fe, (b) Mn, (c) Cu and (d) Zn in 3 soil aggregate size classes (<0.25 mm, 0.25-2 mm, >2 mm (mg kg $aggregate^{-1}$). Data are represented as means ± standard error (n=4). Lowercase letters indicate significant differences between N treatments within a soil fraction and water treatment. The capital letters indicate significant differences between soil aggregate sizes across N and water treatments.

Fig. 4 The relationships between soil pH and DTPA-extractable (a) Fe (mg kg aggregate^{-1}), (b) Mn (mg kg aggregate^{-1}), (c) Cu (mg kg aggregate^{-1}) and (d) Zn (mg kg aggregate^{-1}) across all N and water treatments within the soil aggregate size classes.

Fig. ~~S1~~ 5 The relationships between KCl-extractable ammonium (mg kg aggregate^{-1}) and ~~total base cations~~effective cation exchange capacity (ECEC, sum of exchangeable Ca, Mg, K, and Na , cmol kg aggregate^{-1}) within macroaggregates (a) and microaggregates (b) across all N and water inputs.

Base cations and micronutrients in soil aggregates as affected by enhanced nitrogen and water inputs in a semi-arid steppe grassland

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Abstract

The intensification of grassland management by nitrogen (N) fertilization and irrigation may threaten the future integrity of fragile semi-arid steppe ecosystems by affecting the concentrations of base cation and micronutrient in soils. We extracted base cations of exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) and extractable micronutrients of iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) from three soil aggregate sizes classes (microaggregates, <0.25 mm; small macroaggregates, 0.25-2 mm; large macroaggregates, >2 mm) from a 9-year N and water field manipulation study. There were significantly more base cations (but not micronutrients) in microaggregates compared to macroaggregates which was related to greater soil organic matter and clay contents. Nitrogen addition significantly decreased exchangeable Ca by up to 33% in large and small macroaggregates and exchangeable Mg by up to 27% in three aggregates but significantly increased extractable Fe, Mn and Cu concentrations (by up to 262%, 150%, and 55 %, respectively) in all aggregate size classes. However, water addition only increased exchangeable Na, while available Fe and Mn were decreased by water addition when averaging across all N treatments and aggregate classes. The loss of exchangeable Ca and Mg under N addition and extractable Fe and Mn in soil aggregates under water addition might potentially constrain the productivity of this semi-arid grassland ecosystem.

Key words Nitrogen deposition • Irrigation • Calcium • Magnesium • Manganese •

Copper

Introduction

Micronutrient availability constrains net primary productivity (NPP) (Li et al., 2010, 2012), and deficiencies in soil micronutrients, including Fe, Mn, Cu, and Zn, are a problem threatening food production worldwide (Jones et al., 2013). Base cations (i.e., exchangeable Ca, Mg, K, Na) are the predominant exchangeable cations in the calcareous soils (Zhang et al., 2013). They are essential for soil buffering capacity particularly in soils affected by atmospheric acid deposition (Lieb et al., 2011), serve as good indicators of soil fertility (Zhang et al., 2013), and are critical nutrients for both plant and microbial metabolism (Cheng et al., 2010). For instance, Ca regulates plant cell signaling, cell division, and carbohydrate metabolism (McLaughlin and Wimmer, 1999), and Mg is important for photosynthesis and energy storage (Lucas et al., 2011). Biogeochemical processes may be driven by base cation and micronutrient supply; for instance, root-surface phosphatase activity is correlated with available Ca and Mg (Gabbrielli et al., 1989) and Mg and Zn availability are important for litter decomposition (Powers and Salute, 2011).

The availability of base cations and micronutrients is influenced by environmental changes, such as altered N and water availability (Treseder, 2008). The availability of base cations varies with edaphic properties, such as soil pH (Katou, 2002), organic matter fractions (Oorts et al., 2003) and soil particle sizes (Beldin et al., 2007). Prolonged N inputs generally causes soil acidification and subsequent losses of soil cations (McLaughlin and Wimmer, 1999; Cheng et al., 2010), and micronutrient availability may increase under soil acidification (Malhi et al., 1998) causing toxicity to both plants and soil microorganisms in extreme cases (Bowman et al., 2008; Horswill et al., 2008). Changes in precipitation regime and soil moisture levels may interact with inorganic N affecting soil microbial activities (Wang et al., 2015a) including the decomposition of soil organic matter (SOM) and nutrients release and their subsequent transport in the soil (Dungait et al., 2012; Nielsen and Ball, 2014). In sandy soils, increased precipitation might promote leaching of nitrate and counter-ions (such as base cations) (Ochoa-Hueso et al., 2014).

Soil aggregate structure predominantly controls SOM dynamics (Six et al., 2004) and microbial activities (Dorodnikov et al., 2009), and soil aggregate stability can serve as an indicator for grassland ecosystem health (Reinhart et al., 2015). In comparison with macroaggregates, microaggregates provide preferential sites for soil C stabilization (Wang et al., 2015b) and the SOM herein is more microbial-processed as evidenced by natural abundance stable ^{13}C values (Gunina and Kuzyakov, 2014; Wang et al., 2015b). More microbial-processed SOM (i.e. more functional groups) and potentially higher mineral contents within microaggregates (Creamer et al., 2011) would purportedly provide more binding sites for base cations and micronutrients. Therefore, dynamics of soil base cations and micronutrients in aggregate scale would be a good indicator for soil health and for the potential of metal nutrients sustainability. However, studies concerning aggregate-scale distribution of base cations and micronutrients under enhanced N input and precipitation are still rarely seen.

Semi-arid steppe grasslands support diverse animal and plant species (Kang et al., 2007) and are experiencing or will experience enhanced atmospheric nitrogen (N) deposition and precipitation (Niu et al., 2009; Bai et al., 2010; Zhang et al., 2014). Previously, we demonstrated that water addition promoted the incorporation of microaggregates into macroaggregates and enhanced decomposition rates within microaggregates compared to macroaggregates, and that the addition of N depressed extracellular enzyme activities within soil aggregates as a result of soil acidification, after 9-years in a field experiment in the semi-arid steppe grasslands of Inner Mongolia (Wang et al., 2015a,b). In this study, we investigated the changes of base cations and micronutrients within the soil aggregates. We hypothesized that (1) both concentrations of base cations and micronutrients would increase in microaggregates because of the increased abundance of adsorption sites provided by greater SOM and mineral contents therein, and (2) that increased N and water inputs would decrease base cations and increase the availability of micronutrients within soil aggregates due to soil acidification and leaching in the free-draining soil.

Materials and methods

Study sites and experiment design

The experiment was conducted in the Inner Mongolia Restoration Ecological Research Station (IMRERS) in the south of Duolun County, Inner Mongolia, China (42°02′27″N, 116°17′59″E, elevation 1,324 m a.s.l). The topography of the experimental area is flat. The mean annual temperature is 2.1 ºC, ranging from -17.8 ºC in January to 18.8 ºC in July, and mean annual precipitation is 379.4 mm (Xu et al., 2012). The soil is a Haplic Calcisols according to the FAO classification (IUSS Working Group WRB, 2015) with a texture of sandy loam (0-10 cm): 63% sand, 20% silt, and 17% clay (Wang et al., 2014). The chemical characteristics of the 0 – 10 cm depth of whole soil are given in Wang et al. (2014).

The experimental design is described in detail in Wang et al. (2014). Briefly, experimental plots were set up and run for 9 years. A split-plot design was applied with water and N addition being the two treatments. In April 2005, twelve 8 m × 8m plots were established in each of seven treatment blocks with 1 m buffer zone between any two adjacent plots. Each block was divided into two main plots with either ambient precipitation or 180 mm water addition) as treatments. Each main plot was divided into six subplots and nitrogen treatments (urea pellets) were applied to a randomly selected subplot (dispersed on the top of the soil) (Xu et al., 2012). Two subplots were phosphorus addition treatments which were not considered in this study. Additional water (approximately 50% of mean annual precipitation) was added to the water addition plots by sprinkling 15 mm weekly during the middle of the growing season from June to August as over 65% of annually total precipitation occurs during this time. The chemical composition of the irrigation water was listed as Table S1. Nitrogen additions were split applications with half applied in early May and the other half in late June at the rates of 0 (control plots, defined as CK), 5 (N_5), 10 (N_{10}), and 15 (N_{15}) g N m^{-2} yr^{-1} (Xu et al., 2012). The background N inputs (atmospheric deposition plus fertilizer application) in this area are about 5 g N m^{-2} yr^{-1}, so these manipulations represent 100%, 200% and 300% surplus of nitrogen compared to the background N inputs (Wang et al., 2015a).

Soil sampling and aggregate-size fractionation

In September 2013, top soils (0 – 10 cm) were sampled by compositing five randomly selected cores within each plot from four out of seven blocks. Fresh soil samples were stored at 4ºC during transportation to the laboratory. Soil aggregates were isolated by a dry-sieving method according to Dorodnikov et al. (2009) to minimize the disruption in microbial activities and to prevent leaching of available nutrients by wet sieving. Briefly, fresh bulk soil samples (gravimetric water content of 10-15%) were gently passed through a 5 mm screen and transferred to a nest of sieves (2 and 0.25 mm) on a mechanical shaker Retsch AS200 Control (Retsch Technology, Düsseldorf, Germany). The sieves were mechanically shaken (amplitude 1.5 mm) for 2 min to separate the aggregates > 2 mm (large macroaggregates), 2 – 0.25 mm (small macroaggregates), and < 0.25 mm (microaggregates). The mass proportions of large macro-, small macro-, and microaggregates were 30.3-44.8%, 38.3-42.8%, and 15.3-31.1%, respectively (Wang et al., 2015b). No correction was made for sand because sand grains can be completely embedded within macroaggregates. The chemical characteristics of bulk soil and soil aggregates (0-10 cm) are given in Table 1 (Wang et al., 2014, 2015a,b).

Soil physiochemical and biological parameters

Soil aggregate pH and electrical conductivity (EC) was determined in a 1:5 (w/v) soil-to-water extract with a PHS-3G digital pH meter (Precision and Scientific Corp., Shanghai, China) and a Orion 150A+ conductivity meter (Thermo Fisher Scientific Inc., Beverly, USA), respectively. Soil organic carbon (SOC) within aggregate classes was determined for acid-fumigated soils (to remove carbonates) using elemental analyzer (EA1112, ThermoFisher Scientific, Japan). Concentration of soil aggregate ammonium was determined colorimetrically from 2 M KCl soil extracts (Wang et al., 2015b).

Extraction and analysis of base cations and micronutrients

Soil aggregate base cations were extracted and analysed using the method of Ochoa-Hueso et al. (2014). Briefly, 2.5 g of each soil aggregate fraction was extracted by 50 ml 1 M ammonium acetate (CH_3COONH_4) (pH 7.0). The slurry was orbitally shaken for 30 min at 150 rpm and then filtered through Whatman no. 2V filter paper (quantitative and ash-free). Micronutrients (Fe, Mn, Cu, Zn) within soil aggregates were extracted by diethylenetriamine pentaacetic acid (DTPA) (Lindsay and Norvell, 1978). 10 g of soil aggregates was weighted into Erlenmeyer flask to mix with 20 mL 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA (triethanolamine) (pH 7.0). The mixture were shaken at 180 rpm for 2 h and filtered. All of the extractable metal cations were analyzed by atomic absorption spectrometer (AAS, Shimadzu, Japan). The sum of exchangeable Ca, Mg, K, and Na was defined as effective cation exchange capacity (ECEC).

Statistical analyses

Three-way ANOVAs with a split-plot design were used to test the effect of water (between subject), N addition (within subject) and soil aggregate sizes on electrical conductivity (EC), the concentrations of base cations (exchangeable Ca, Mg, K, and Na) and available micronutrients (Fe, Mn, Cu, and Zn). Multiple comparisons with Duncan design were performed to determine differences in EC, base cations, and available micronutrients among N rates and run separately for ambient and enhanced precipitation within each soil aggregate. Pearson correlation analysis was used to examine the relationships between base cations and available micronutrients. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, U.S.A.) and statistical significance was accepted at $P<0.05$.

Results

Responses of electrical conductivity (EC) to N and water addition

Under ambient water conditions, soil EC was significantly reduced in N addition plots from 272.5 to 179.2 μs cm^{-1} in large macroaggregates, from 249.4 to 188.6 μs cm^{-1} in small macroaggregates, and from 295.3 to 173.9 μs cm^{-1} in microaggregates, except for N_5 in large macro- and microaggregates as compared to the control (CK) (Fig. 1a, Table 2). Under elevated water conditions, EC values were significantly smaller in N_{15} plots across soil aggregate size classes (Fig. 1a). The N_{15} treatment significantly decreased soil EC from 237.8 to 194.5 μs cm^{-1} in large macroaggregates, from 247.5

to 192.9 μs cm^{-1} in small macroaggregates, and from 293.5 to 192.7μs cm^{-1} in microaggregates (Fig. 1a). Soil EC values were significantly larger in water addition plots at N application rates of N_{10} in large macroaggregates, N_5 and N_{10} in small macroaggregates, and N_{10} in microaggregates comparing to respective N plots of ambient precipitation (Fig. 1a).

Response of ECEC, exchangeable Ca, Mg, K, and Na and DTPA-extractable micronutrients to N and water addition

The ECEC (sum of exchangeable Ca, Mg, K, and Na) in soil aggregates increased in the order: small macroaggregates < large macroaggregates < microaggregates averaging across all N and water treatments (Table 2, Fig. 1b). Soil base cations decreased with N addition in both large and small macroaggregates under both ambient and elevated water conditions (Fig. 1b).

When averaging all the N and water treatments, the greatest concentrations of exchangeable Ca, Mg, and K were all detected in microaggregates, followed by large macroaggregates (Fig. 2a, b, c). Microaggregates contained significantly more exchangeable Na than the large macroaggregates averaging across all the treatments (Fig. 2d). Under ambient precipitation, exchangeable Ca was significantly less in N-addition plots with N_5, N_{10}, and N_{15} in large macroaggregates, and in N_{10} and N_{15} plots in small macroaggregates as compared to control plots by as much as 33 % (between CK and N_{15} treatments) (Fig. 2a). Under elevated water conditions, N addition significantly decreased exchangeable Ca in both large (N_5, N_{10}, and N_{15}) and small macroaggregates (N_{10} and N_{15}) by as much as 26 % (Fig. 2a). Under ambient water conditions, exchangeable Mg was significantly lower in N_{15} in large macroaggregates, in N_5 and N_{15} in small macroaggregates, and in N_{15} in microaggregates by 4 - 27 % (Fig. 2b). Under increased water inputs, N addition decreased exchangeable Mg in N_{10} and N_{15} plots in both large and small macroaggregates and in N_5, N_{10}, and N_{15} in microaggregates by 2 - 19 % (Fig. 2b). Both exchangeable K and Na showed no response to N addition across all aggregate-size classes (Fig. 2c, d). Significant positive water effects were only seen on the concentration of exchangeable Na when comparing the means of all N treatments and aggregate classes between ambient precipitation and water addition (Table 2).

DTPA-extractable micronutrients were distributed differently across aggregate-size classes (Fig. 3, Table 2). Significantly less extractable Fe and Mn were detected within small macroaggregates compared to large macro- and microaggregates (Fig. 3a, b). Extractable Cu increased in the order large macro- > micro- > small macroaggregates when averaging all the N and water treatments (Fig. 3c). The largest extractable Zn was observed in microaggregates, while no significant difference was found for Zn between large and small macroaggregates (Fig. 3d). Following N additions, DTPA-extractable Fe, Mn, and Cu significantly increased within all soil aggregates under both ambient and elevated water conditions by as much as 262 %, 150 %, and 55 %, respectively (Fig. 3a, b, c). No overall N effects were detectable on extractable Zn (Table 2), but Zn decreased significantly with N_{10}

and N_{15} treatments under water addition within microaggregates (Fig. 3d). Extractable Fe and Mn significantly decreased with water addition under N addition rates of 5, 10, and 15 g N m^{-2} yr^{-1} within large macroaggregates (Fig. 3a,b, Table 2).

Correlations between soil physicochemical parameters, base cations and micronutrients

Significant positive correlations were observed between SOC and soil exchangeable Ca, Mg, and K, and between pH and EC, exchangeable Ca, Mg, and Na (Table 3). There were significant positive relationships between exchangeable Ca vs. Mg, Ca vs. K, Ca vs. K, Mg vs. K, and Mg vs. Na (Table 3). Micronutrients Fe, Mn, and Cu were negatively correlated with soil pH across all soil aggregate size classes (Fig. 4a, b, c). Within macroaggregates, ECEC significantly and negatively correlated with extractable ammonium (Fig. 5a).

Discussion

Distribution of base cations and micronutrients within soil aggregates

Relatively increased clay mineral and SOM contents are commonly recognized to be associated with the smallest aggregate size fractions (< 0.25 mm), both of which play an essential role in retaining soil base cations especially in sandy, free-draining soils similar to our experimental field plot (Oorts et al., 2003; Beldin et al., 2007). In line with our initial hypothesis, a significant positive correlation was detected between SOC (the largest component of SOM) and exchangeable Ca, Mg, K, and ECEC (Table

3), and their distribution was similar to the distribution pattern of SOC and total nitrogen with the highest concentrations in microaggregates (Wang et al., 2015b). Soil organic matter was more decomposed within microaggregates (Wang et al., 2015b) and thus may have provided more binding sites (e.g. more carbonyl functions; Golchin et al., 1994) for exchangeable base cations. It is widely reported that available micronutrients correspond to both SOM and clay content which are both usually more abundant in microaggregates (e.g. Sharma et al., 2004; Six et al., 2004). Although we observed greater available Zn in microaggregates compared to macroaggregates, there was no significant relationship of SOC with Fe, Mn, and Cu, which had quite different distribution patterns from SOC within soil aggregate-size classes and was similar to the findings by Głąb and Gondek (2014) in bulk soil. Due to the fact that large macroaggregates are responsible for providing nutrients for plants and the early stages of SOM formation (Six et al., 2004), plant cycling (uptake and biomass decomposition) might contribute to the higher concentrations of Fe, Mn and Cu in large macroaggregates (Jobbágy and Jackson, 2001).

Response of EC and base cations to nitrogen and water addition

Protons released when soils acidify may exchange with base cations making them available for take-up by plants or transport (and loss) by water in soils (Lucas et al., 2011; Malhi et al., 1998; Sharma et al., 2004). As EC represents salt concentration in soil solution (Zhou et al., 2011), significantly lower EC values might suggest the loss of ions, in line with the decline of ECEC under higher N rates (Fig. 1a vs. Fig. 1b).

Deficiencies in soil base cations caused by N deposition could pose a threat to ecosystems through their effect on soil microbial activity (Vitousek et al., 1997; Treseder, 2008). Many studies on the effects of chronic N addition to soil contribute shifts in soil microbial community structure and activity to the reduction of soil pH (Ramirez et al., 2010; Wang et al., 2014), and we previously reported soil enzyme depression under N addition attributed to soil acidification (Chung et al., 2007; Wang et al., 2014). Consistent with our hypothesis, we observed a decrease in Ca and Mg, which are the major base cations in macroaggregates (Fig. 2). This decrease corresponded to a negative correlation between NH_4^+ and ECEC across soil macroaggregates (Fig. 5a). Nitrogen addition increased NH_4^+ concentrations across all three soil aggregate size classes (Wang et al., 2015b) which may displace soil aggregate base cations and bind strongly to soil surfaces (Matschonat and Matzner, 1996). We did not measure leaching rates in the field experiment, but these are likely to be substantial given the sand content of the soil (> 60%). Increased leaching under water addition that caused NO_3^- loss was previously reported (Wang et al., 2015b) and may indicate a route for the loss of Ca and Mg within soil aggregates, as observed by Currie et al. (1999) and Lucas et al. (2011). Our results contradict those of Ochoa-Hueso et al. (2014) who reported an increase of extractable base cations under N deposition in a highly buffered, limestone soil in the semi-arid Mediterranean region. However, where N addition increases NPP in an N-limited soil, such as those of semi-arid steppe grasslands (Xu et al., 2014) a coincident uptake of base cations by vigorous plant growth may occur (Tomlinson, 2003). But we did not investigate changes in the cation content of plant tissues in this experiment to confirm this flux. Furthermore, no effect of N addition was observed on soil aggregate K and Na concentrations (although Ca and Mg decreased; Fig. 2, Table 2) indicating the selective depletion of base cations (Lu et al., 2014). The depletion of base cations within soil aggregate can accelerate a reduction in the buffering capacity of soils and accelerate soil acidification (with increased N at ambient water soil pH 6.97 decreased to 6.10, and with added water, 7.17 to 6.42; Wang et al., 2015a); the dynamics of the base cations were affected substantially. Water addition increased EC and exchangeable Na but did not affect the other base cations (Table 2; Fig. 1a, 2). The positive effect of water on soil EC and exchangeable Na might be caused by the relatively high EC value and Na concentration in irrigative water in this calcareous grassland (Table S1). We previously reported that water addition increased soil microbial activity at the study site (Wang et al., 2014, 2015a): if litter decomposition rates were promoted as a consequence (e.g. Liu et al., 2006) Ca, Mg and K may have been released from this secondary biological source to supplement the soil pools.

Response of micronutrients to nitrogen and water addition

Micronutrient cycling relies on the balance between input (mineral weathering release), recycling (plant biomass turnover), retention (sorption reactions), and removal (biomass harvesting, fire, and hydrologic leaching) (Li et al., 2008). Long-term N addition caused large increases in extractable Fe, Mn and Cu concentrations in all soil aggregate size classes (by up to 262%, 150%, and 55 %, respectively; Fig. 3a, b, c), presumably due to the decrease in soil pH which may increase micronutrient release through weathering and desorption from soil minerals and SOM (Malhi et al., 1998). Acidification caused by the long-term addition of N negatively affected microbial activity previously observed as the depression of enzyme activity in these experimental soils (Chung et al., 2007; Wang et al., 2014). The mechanism underlying this biological response was not explored but is likely to be complex and due in part to the effect of the changes in micronutrient supply. Long-term irrigation had the opposite effect on extractable Fe and Mn within soil aggregates where increases in soil aggregate pH (which usually decrease the mobilization of micronutrients) were detected compared to ambient precipitation plots (Wang et al., 2014). Our previous study suggested that water addition increased soil pH from 7.1 to 7.3 in CK and from 5.6 to 6.1 in N_{15} plots for large macroaggregates, from 7.1 to 7.5 in CK and from 5.8 to 6.2 in N_{15} plots for small macroaggregates, and from 7.1 to 7.4 in CK and from 5.9 to 6.2 in N_{15} plots for microaggregates (Wang et al., 2015). A previous study carried out at the same plots showed that water addition significantly increased NPP (Xu et al., 2010), suggesting enhanced uptake of growing plants may have reduced concentrations of Fe and Mn in the soil aggregates. This is consistent with the findings of Li et al. (2008) who reported that Mn and boron uptake by plants outpaced their resupply by mineral weathering. However, Fe and Mn may be toxic to plants (Brown and Jones, 1977), so losses by increased leaching may be the most likely route for their decrease in the irrigated plots. However, neither leaching nor plant performance was measured in this experiment.

Conclusions

Consistent with our first hypothesis, microaggregates had greater base cation concentrations, which was ascribed to a proportion of sorption provided by increased SOM and clay contents associated with the smallest aggregate size class. However, extractable micronutrients (except Zn) did not follow the same concentration pattern as base cations across three soil aggregate size classes. Under N addition, exchangeable Ca and Mg decreased while extractable micronutrients (Fe, Mn and Cu) increased, which was related to the slight but apparently important decrease in soil pH. Water addition showed no impact on exchangeable Ca, Mg and K, but decreased available Fe and Mn averaging across soil aggregates which partially supported our second hypothesis. Our findings suggest that uncontrolled or long-term N fertilization and irrigation of semi-arid steppe soils cause significant changes in the concentrations of base cations and micronutrients which will ultimately decrease soil fertility, constrain NPP and the nutrient quality of plants. The pathway of the losses (e.g. leaching or uptake into plant tissues followed by harvest) need to be determined in order to manage large and fragile semi-arid grassland systems.

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Tables

Table 1 The chemical characteristics of bulk soil and soil aggregates without field-manipulated treatments. SOC, TN, C/N, and DOC represent soil organic carbon, total nitrogen, the ratio of SOC to TN, and dissolved organic carbon, respectively. Data are represented as means ± standard error.

		Soil aggregates (mm)		
	Bulk soil	> 2	0.25-2	< 0.25
SOC (g kg soil^{-1})	18.9±1.7	18.2 ± 1.0	15.1 ± 2.0	21.6 ± 1.4
TN (g kg soil^{-1})	1.8±0.2	1.9 ± 0.1	1.5 ± 0.2	2.2 ± 0.1
C/N	10.4±1.3	9.5 ± 0.5	10.4 ± 0.4	10.0 ± 0.2
pH	7.0±0.2	7.1 ± 0.2	7.1 ± 0.2	7.1 ± 0.3
DOC	69.8±5.6	72.5±6.8	66.6±1.3	71.2±1.5
NO_3^--N (mg kg soil^{-1})	27.3±5.9	4.2 ± 0.1	31.6 ± 2.2	34.4 ± 3.8
NH_4^+-N (mg kg soil^{-1})	9.4±1.6	17.0 ± 1.3	17.9 ± 2.5	22.9 ± 1.0

Data from Wang et al. (2014, 2015a,b)

Table 2 Results (*F* values) of ANOVAs with a split-plot design on the effects of N, water addition (W), and soil fractions (S) on soil electrical conductivity (EC), exchangeable Ca, Mg, K, Na, effective cation exchange capacity (ECEC, sum of exchangeable Ca, Mg, K, and Na , cmol kg aggregate^{-1}), and available Fe, Mn, Cu, and Zn (mg kg aggregate^{-1}).

	EC	Ca	Mg	K	Na	ECEC	Fe	Mn	Cu	Zn
W	11.60*	3.07	5.08	0.17	15.6**	4.03	27.8**	14.1*	2.40	3.92
N	14.20**	6.36**	38.7**	0.33	1.71	7.36**	147**	136.3**	44.7**	2.47
S	3.63*	25.0**	168.3**	123.7**	3.49*	30.7**	17.7**	30.1**	33.7**	33.8**
W×N	6.90**	1.01	5.68**	6.81**	1.26	1.25	2.36	3.40*	7.23**	4.34**
W×S	0.28	1.03	3.75*	0.98	0.34	1.03	6.07**	10.8**	6.60**	2.32
N×S	0.77	0.87	0.36	0.88	1.81	0.88	1.59	2.43*	0.85	2.04
W×N×S	0.86	0.44	1.03	1.41	0.61	0.42	0.99	2.54*	1.16	3.84**

* and ** indicating the significant level at P<0.05, 0.01, respectively

Table 3 Correlation analyses (*R* values) among exchangeable base cations, effective cation exchange capacity (ECEC), soil organic carbon (SOC), soil pH, and soil electrical conductivity (EC).

	pH	EC	Ca	Mg	K	Na	ECEC
SOC	0.11	0.19	0.42**	0.73**	0.81**	0.17	0.46**
pH		0.66**	0.35**	0.48**	0.04	0.33**	0.37**
EC			0.28**	0.40**	0.15	0.16	0.29**
Ca				0.60**	0.53**	0.23*	0.998**
Mg					0.77**	0.24*	0.65**
K						0.10	0.57**
Na							0.26*

* and ** indicating the significant level at P<0.05, 0.01, respectively

Table S1 Chemical characteristics of the irrigation water.

	Irrigation water
EC (us cm^{-1})	196.1±6.4
pH	7.7±0.07
Ca (mmol L^{-1})	1.6±0.03
Mg (mmol L^{-1})	0.8±0.02
K (mmol L^{-1})	0.04±0.002
Na (mmol L^{-1})	0.6±0.02

The symbol ‘-’indicate the ions were not detected.

Figure Legends

Fig.1 The effect of 9-year of water (ambient vs. water addition) and N additions (0 [CK], 5 [N_5], 10 [N_{10}], 15 [N_{15}] g N m^{-2} yr^{-1}) on (a) electrical conductivity (EC, us cm^{-1}) and (b) effective cation exchange capacity (ECEC, sum of exchangeable Ca, Mg, K, and Na, cmol kg $aggregate^{-1}$). Data are represented as means ± standard error (n=4). Lowercase letters indicate significant differences between N treatments within a soil aggregate class size and water treatment. The capital letters indicate significant differences between soil aggregate sizes across N and water treatments.

Fig.2 The effect of 9-year of water (ambient vs. water addition) and N additions (0 [CK], 5 [N_5], 10 [N_{10}], 15 [N_{15}] g N m^{-2} yr^{-1}) on exchangeable (a) Ca, (b) Mg, (c) K and (d) Na concentrations (cmol kg $aggregate^{-1}$) in 3 soil aggregate size classes (<0.25 mm, 0.25-2 mm, >2 mm). Data are represented as means ± standard error (n=4). Lowercase letters indicate significant differences between N treatments within a soil aggregate size class and water treatment. The capital letters indicate significant differences between soil aggregate sizes across N and water treatments.

Fig. 3 The effect of 9-years of water (ambient vs. water addition) and N additions (0 [CK], 5 [N_5], 10 [N_{10}], 15 [N_{15}] g N m^{-2} yr^{-1}) on DTPA-extractable micronutrients (a) Fe, (b) Mn, (c) Cu and (d) Zn in 3 soil aggregate size classes (<0.25 mm, 0.25-2 mm, >2 mm (mg kg $aggregate^{-1}$). Data are represented as means ± standard error (n=4). Lowercase letters indicate significant differences between N treatments within a soil fraction and water treatment. The capital letters indicate significant differences between soil aggregate sizes across N and water treatments.

Fig. 4 The relationships between soil pH and DTPA-extractable (a) Fe (mg kg aggregate^{-1}), (b) Mn (mg kg aggregate^{-1}), (c) Cu (mg kg aggregate^{-1}) and (d) Zn (mg kg aggregate^{-1}) across all N and water treatments within the soil aggregate size classes.

Fig. 5 The relationships between KCl-extractable ammonium (mg kg aggregate^{-1}) and effective cation exchange capacity (ECEC, sum of exchangeable Ca, Mg, K, and Na , cmol kg aggregate^{-1}) within macroaggregates (a) and microaggregates (b) across all N and water inputs.

Fig. 1

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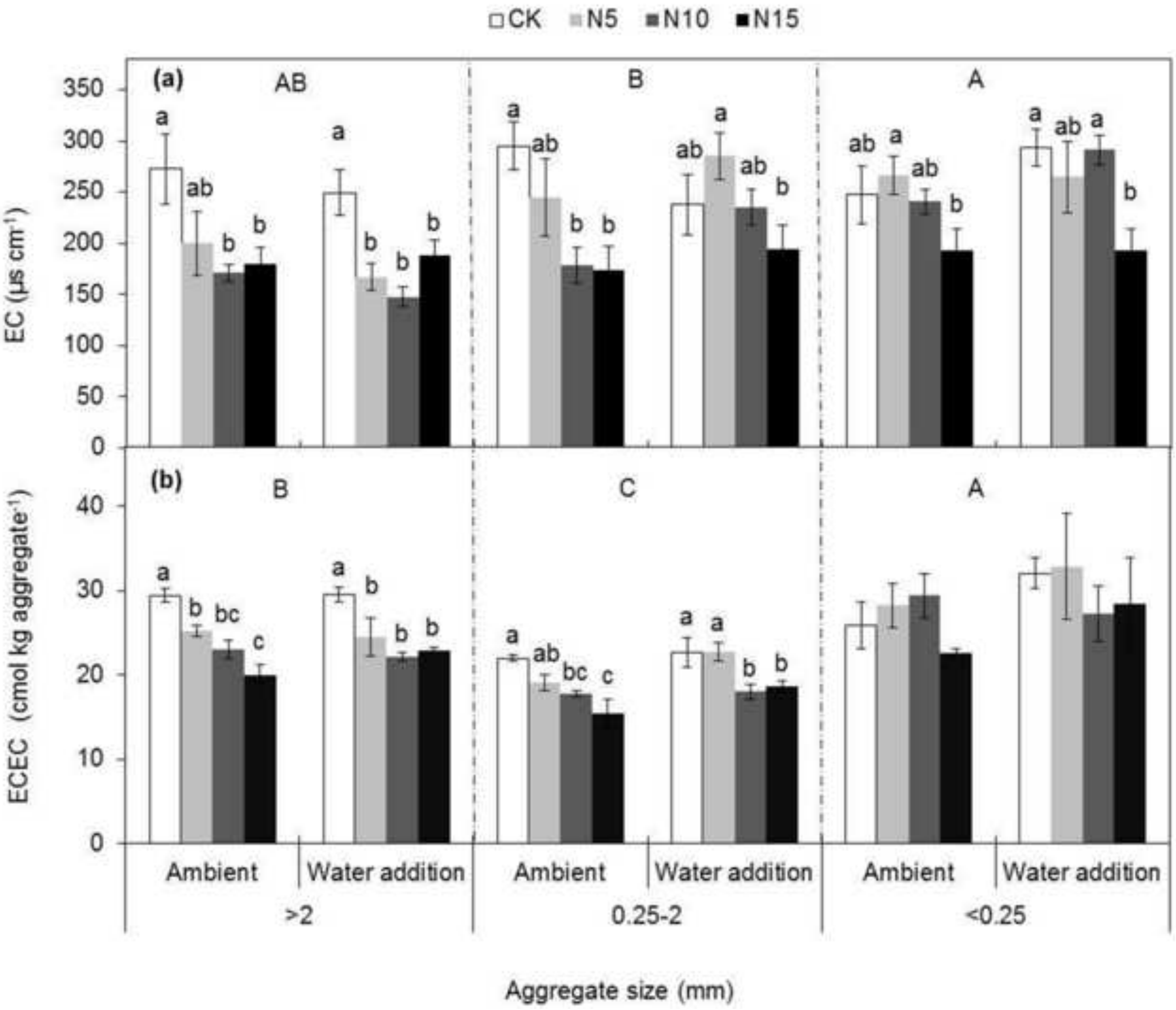

Fig. 2

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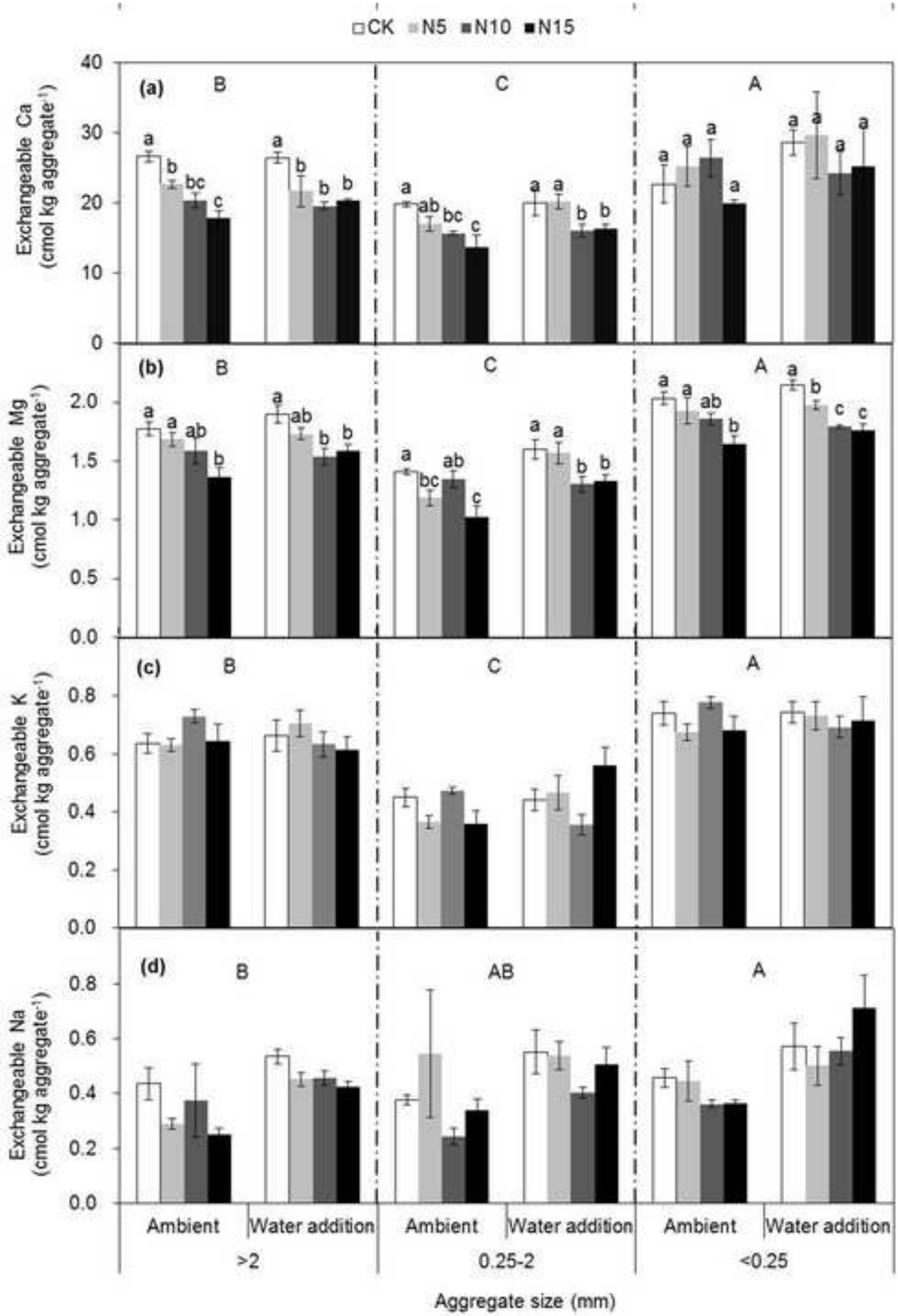

Fig. 3

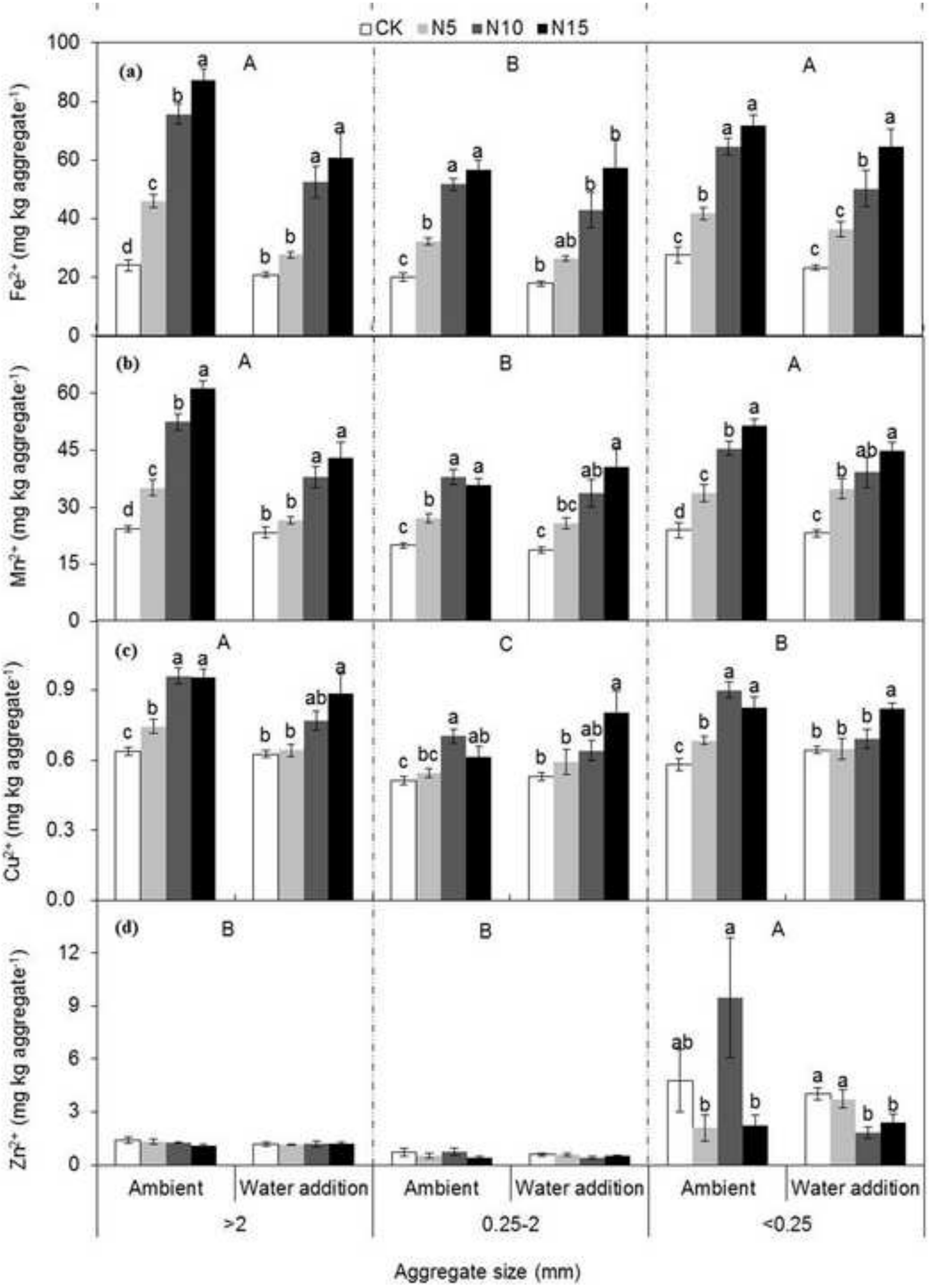
□CK ■N5 ■N10 ■N15
(a)
Fe^{2+} (mg kg aggregate^{-1})
0
20
40
60
80
100
A
B
A
d c b a
b b a a
c b a a
b ab b b
c b a a
c c b a
(b)
Mn^{2+} (mg kg aggregate^{-1})
0
15
30
45
60
A
B
A
d c b a
b b a a
c b a a
c bc ab a
d c b a
c b ab a
(c)
Cu^{2+} (mg kg aggregate^{-1})
0.0
0.3
0.6
0.9
A
C
B
c b a a
b b ab a
c bc a ab
b b ab a
c b a a
b b b a
(d)
Zn^{2+} (mg kg aggregate^{-1})
0
3
6
9
12
B
B
A
ab b a b
a a b b
Ambient
Water addition
Ambient
Water addition
Ambient
Water addition
>2
0.25-2
<0.25
Aggregate size (mm)

Fig. 4

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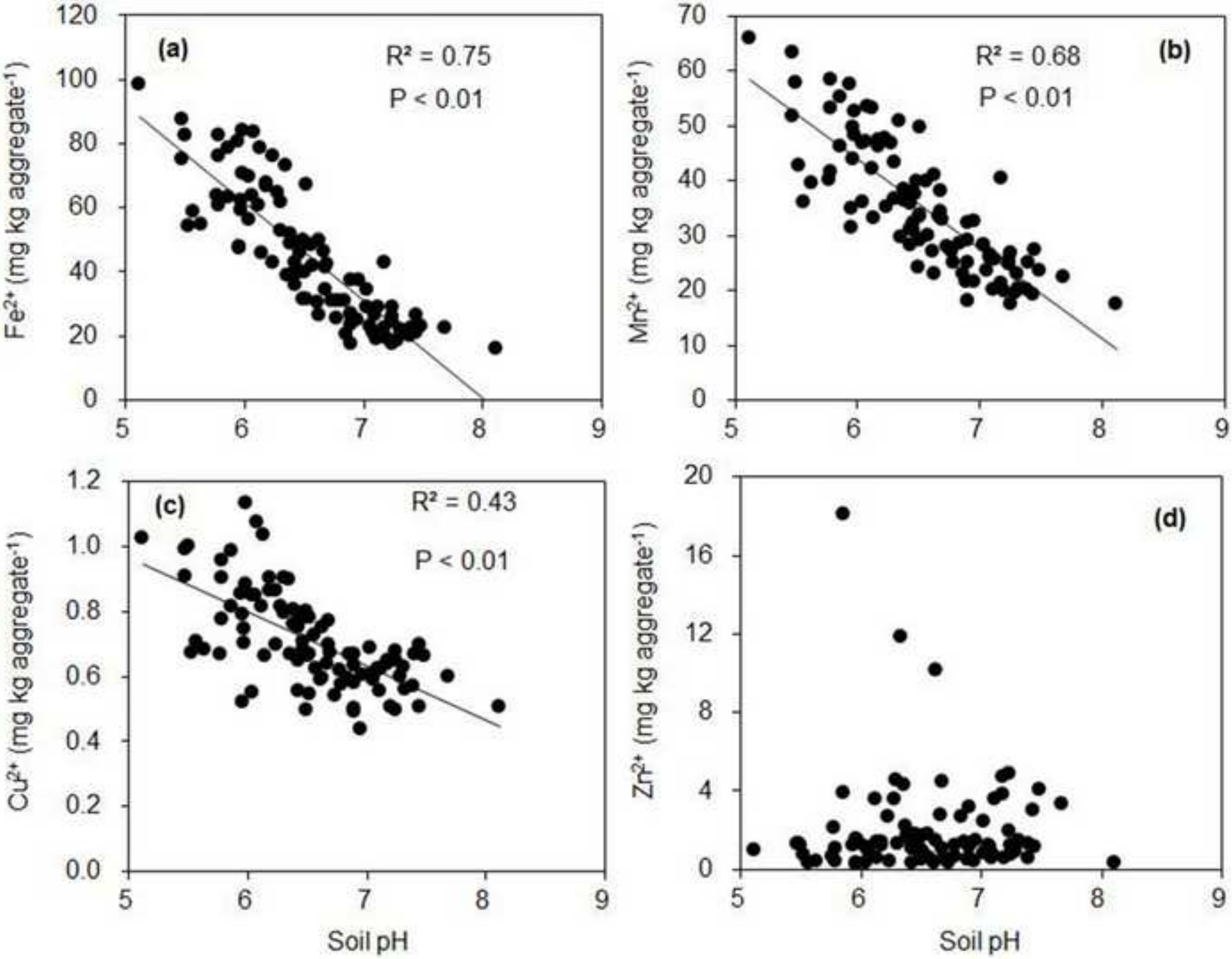

Fig. 5

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