Carbon and nitrogen storage in particle-size fractions of topsoil along a 3000 km aridity gradient in northern China

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15 Abstract.

Climate factors such as aridity significantly influence soil carbon (C) and 16 17 nitrogen (N) storage dynamics in terrestrial ecosystems. Further, soil composition plays an important role in driving changes of soil C and N storage at regional scale. 18 19 However, it remains uncertain whether such changes result from the variation of 20 different soil particle-size factions or the C and N concentrations in those fractions. 21 We examined the distribution of total C and N in both bulk soil and different soil 22 particle-size fractions, including sand (53-2000 μ m), silt (2-53 μ m) and clay (<2 μ m), 23 along a 3000 km transect in arid and semi-arid grasslands of northern China. Across 24 the whole transect, sand content was positively and silt content was negatively correlated with increasing aridity. The total C and N concentrations and storage in 25 26 bulk soils as well as in the three particle-size fractions tended to be negatively correlated with aridity. The concentrations and storage of total C and N in bulk soils 27 were positively correlated with silt and clay contents and negatively correlated with 28 29 sand content. There were positive correlations between the concentrations and storage of C or N in bulk soils and the C or N concentrations in the three soil particle-size 30 31 fractions. By characterizing such a large scale aridity gradient, our results highlight that aridity would decrease soil C and N storage both by favoring increased sand 32 content and by decreasing C and N concentrations in all the three soil fractions. These 33 patterns thus have significant implications for understanding soil C and N 34 sequestration under scenarios of increasing aridity in global drylands that are 35 predicted to occur this century. 36

37 Keywords soil particle-size fractionation, soil organic matter, temperate steppe,
38 carbon sequestration, precipitation to evapotranspiration, drought

39 **1 Introduction**

Grasslands, which cover nearly 40% of the world's land area, store approximately 40 41 one-third of the total C in terrestrial ecosystems (He et al., 2012; White et al., 2000). 42 Consequently, C turnover in grassland soils is considered to be a critical component 43 of the global C cycle (Fisher et al., 1994; Wang et al., 2009). In China, grasslands 44 account for 41.7% of terrestrial land area and are mainly distributed in arid and 45 semi-arid regions (NSBC, 2002). Carbon and N cycling in the grasslands of northern China are relatively sensitive to global change factors, such as increases in drought, 46 47 more extreme precipitation regimes, and global warming (Wang et al., 2014; Song et 48 al., 2012). However, this sensitivity may show regional variations due to variability in both climate and soil characteristics. Greater understanding of the controls on regional 49 variation of soil C and N stocks in Chinese grasslands would facilitate projections of 50 51 regional C and N cycling under global change scenarios.

52 Soil C and N storage in grassland ecosystems are closely correlated with climatic 53 conditions. In arid and semi-arid ecosystems, soil C and N storage are positively 54 correlated with mean annual precipitation (MAP) at the regional scale (He et al., 2014; 55 Nichols, 1984). This positive relationship is driven by the fact that water availability 56 is the dominant limiting factor for plant growth (and thus soil organic matter inputs) 57 in these ecosystems. In contrast, mean annual temperature (MAT) is negatively 58 correlated with soil C and N storage, as higher temperature generally enhances

microbial decomposition more than detrital production (Homann et al., 2007; Miller
et al., 2004; Schimel et al., 1994). Previous studies found that soil C and N tends to
decrease with increasing aridity (that is the degree of dryness of the climate at a given
location) largely due to decreased primary productivity (Carrera and Bertiller, 2010;
Delgado-Baquerizo et al., 2013; Sanaullah et al., 2014).

Variation in soil particle-size fractions also exerts significant controls on the 64 storage and turnover of soil organic matter (SOM) (Chen et al., 2010; Christensen, 65 66 2001; Qin et al., 2010) and increasing attention has focused upon the responses of C 67 and N pools in different soil particle-size fractions to climate change (Amelung et al., 1998; He et al., 2014). Clay and silt fractions in soil usually have higher C and N 68 concentrations and storage than that of sand fraction, so soils with higher clay and silt 69 70 contents generally have higher soil organic C (SOC) and N storage (Amelung et al., 71 1998; Feller and Beare, 1997; Follett et al., 2012; Hassink, 1997). This pattern reflects that organic materials are preferably decayed from pools of coarse soil particles; these 72 73 relatively C- and N-rich decomposition products tend to accumulate in finer clay and silt particles (Amelung et al., 1998). Moreover, clay and silt may physically protect 74 75 organic materials from decomposition and promote the accumulation of recalcitrant material in the fine particle-size fractions of soils (Hassink, 1997; Zhao et al., 2006; 76 Chen and Chiu, 2003). 77

Aridity, which is intensified by decreasing MAP and increasing MAT, is projected to increase in drylands worldwide during this century (Dai, 2013; Delgado-Baquerizo et al., 2013); this change may significantly diminish soil C and N storage in those

regions (Delgado-Baquerizo et al., 2013; Sanaullah et al., 2014). Variation in soil 81 fraction composition and the C and N concentrations in different soil particle sizes 82 83 may significantly influence the pattern of decreasing bulk soil C and N pools observed with increasing aridity (Amelung et al., 1998; He et al., 2014). However, 84 85 compared with these well-described patterns of variation in soil C and N storage along climate and soil particle size gradients, the relative influence of these factors on 86 terrestrial C and N pools under climate change scenarios such as increasing aridity is 87 88 less clear (Delgado-Baquerizo et al., 2013).

89 To increase our understanding of the variation in both the components of different soil particle-size fractions and their C and N concentrations with increasing aridity at 90 91 the regional scale, we collected soil samples from 58 sites along a 3000 km aridity 92 transect across arid and semi-arid grasslands in northern China. The distinctive features of this transect include a continuum of xeric to mesic grassland types, 93 ascending MAP and descending MAT from the west to the east, complete 94 meteorological records, relatively gentle geographical relief, and light human 95 disturbances (Luo et al., 2015). The objectives of this study were to: (1) examine the 96 97 distribution of C and N in various particle-size fractions of soils across the aridity gradient, and (2) evaluate the relative contributions of soil fraction components and 98 their element concentrations to the changes of soil C and N storage across the aridity 99 gradient. We hypothesized that: (1) concentrations and storage of C and N in soil 100 101 particle-size fractions would be negatively correlated with increasing aridity; and (2) soil C and N storage would decline with increasing aridity due to both an increase in 102

the relative proportion of sand to silt and clay and a decrease of C and Nconcentrations in the three soil fractions.

106 2 Materials and methods

107 **2.1 Study site and soil sampling**

This study was carried out along a 3000 km west-east transect of arid and 108 semi-arid grasslands across Xinjiang, Gansu, and Inner Mongolian in northern China. 109 110 The longitude of the transect ranged from 87 °22'E to 123 °23'E and the latitude ranged 111 from 39 °51'N to 50 °3'N. Along the transect, the MAP increased from 34 mm in west region to 436 mm in east region, whereas the MAT ranged from -5 to 11°C. The 112 aridity (calculated as "1-precipitation / evapotranspiration") of this transect ranged 113 114 from 0.43 to 0.97 and derived from the WorldClim data set (http://www.worldclim.org/) (Hijmans et al., 2005). The main vegetation types were 115 desert steppe, typical steppe, and meadow steppe from the west to east across the 116 transect, with the primary productivity ranging from <10 g m⁻² yr⁻¹ in the desert 117 steppe to >400 g m⁻² yr⁻¹ in the meadow steppe, and showing a negative relationship 118 with aridity (Wang et al. 2014). The species richness per square meter ranged from 0 119 (no plants) in the west of the transect to >30 in the east parts (Lü et al. unpublished 120 data). The soil types were arid, sandy, calcium-rich brown loess, and belonged to 121 Kastanozem soil group in the Food and Agriculture Organization (FAO) classification 122 system (Cheng et al., 2009). 123

Fifty-eight sites were set up along the transect at an interval of 50-100 km. The location and elevation of the sampling sites were measured by GPS (eTrex Venture, Garmin, USA). For each site, one large plot (50 m×50 m) was selected and five subplots (1 m×1 m) were designated within the large plot (the four corners and the 128 center of the large plot). In each subplot, five soil samples (0-10 cm) were randomly 129 collected by a 3.0 cm diameter soil corer and then totally mixed them together as one 130 composite sample which was then sieved through a 2.0 mm sieve. All of the soil 131 samples were returned to the lab and then air dried for further analysis. Soil bulk 132 density (BD) was calculated as the ratio of dry soil mass per unit volume of the 133 sampling core and expressed as g cm⁻³ (Grossman and Reinsch, 2002).

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2.2 Particle-size fractionation

Particle-size fractionation was completed by disrupting soil aggregates of bulk 135 136 soil samples using ultrasonic energy and separating the particle-size fractions by a combination of wet sieving and continuous flow centrifugation (Chen and Chiu, 2003; 137 He et al., 2009). Briefly, 40 g of sieved soil (< 2 mm) following removal of visible 138 139 debris was dispersed in 200 ml of deionized water using a probe-type ultrasonic cell disrupter system (scientz-IID) operating for 15 min in the continuous mode at 361 W. 140 We used a sieve to separate sand (particle size, 53-2000 µm) by manual wet sieving 141 142 method with deionized water. Particles which consisted of silt (2-53 μ m) and clay (< 2 μ m) passing through the sieve during the wet sieving process were collected. In order 143 144 to separate slit from clay, the mixture of particles and water was poured into a 500 ml centrifuge bottles and centrifuged at 682 rpm for 5 min. During this procedure, only 145 the silt fraction sinks to the bottom while the clay fraction remains suspended. The silt 146 fraction was then re-suspended in 200 ml deionized water and re-centrifuged at 476 147 rpm; this procedure was repeated 5 times. The clay fraction was obtained by 148 transferring the suspensions into new centrifuge bottles and centrifuging them at 4000 149

rpm for 30 minutes. All the fractions were dried at 50 $^{\circ}$ C and then ground for further chemical analysis. The concentrations of total C and N in the bulk soil and soil particle-size fractions were determined using an automatic element analyzer (Vario MACRO cube, Elementar Analysensysteme GmbH).

154 2.3 Statistical analysis

All of the relationships between variables were explored by using simple linear 155 regression analyses (58 sites with five subplots as replications in each site). We 156 observed that the relationships were best-fitted by either a first-order equation or a 157 second-order equation. As the contents of sand, silt and clay in soils are not 158 159 independent of each other, stepwise multiple regression analyses, which are highly conservative (Fornara and Tilman, 2008), were used to determine the simultaneous 160 161 effects of soil fraction composition and C and N concentrations in soil particle-size fractions on soil C and N storage. All analyses were performed using SPSS V13.0 162 (SPSS, Chicago, IL, USA). 163 164

165 **3 Results**

166 **3.1 Soil particle-size fractions and BD across the aridity transect**

Sand was the most abundant fraction for most sites, accounting for 21.62-90.65% of the total soil weight along the transect. The content of sand was positively correlated with increasing aridity (Fig. 1). The silt content, which accounted for 4.19-49.29% of the total soil weight, decreased with increasing aridity (Fig. 1). The content of clay was relatively low across the transect, ranging from 1.36-33.7%. There were no significant relationships between clay content and aridity (Fig. 1). Bulk density ranged from 0.90 to 1.72 g cm⁻³ and was positively correlated with aridity.

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175 **3.2 C and N concentrations in bulk soil and different soil particle-size fractions**

Total C (Fig. 2a) and N concentrations (Fig. 2b) in the bulk soil significantly 176 177 decreased with increasing aridity. Soil C concentrations ranged from 2.71 to 50.33 g C kg⁻¹, while the N concentrations ranged from 0.14 to 4.75 g N kg⁻¹. Across the 178 transect, C and N concentrations in all of the three particle-size fractions were 179 180 negatively correlated with increasing aridity (Fig. 2). The total C and N concentrations in the soil particle-size fractions varied greatly among the three soil 181 fractions, with the highest concentrations in clay $(36.06 \pm 1.49 \text{ g C kg}^{-1}, 3.90 \pm 0.17 \text{ g})$ 182 N kg⁻¹) and the lowest in sand $(5.19 \pm 0.56 \text{ g C kg}^{-1}, 0.37 \pm 0.04 \text{ g N kg}^{-1})$ (p < 0.001 183 in both cases). 184

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186 **3.3 C and N storage in bulk soil and different soil particle-size fractions**

187 We found negative correlations between C and N storage in each soil particle-size

fraction and aridity. with the exception of C 188 storage in sand $(y=57.12-161.29x+114.70x^2, R^2=0.48, p<0.0001)$, which first decreased and then 189 increased with increasing aridity (Fig. 3a). Paralleling this pattern, C storage in bulk 190 soils first decreased and then slightly increased with increasing aridity, with the 191 lowest value presented in sites with aridity of ~0.8 ($y=160.49-371.88x+228.73x^2$, 192 $R^2=0.73$, p<0.0001, Fig. 3a). Nitrogen storage in bulk soils was negatively correlated 193 with aridity (Fig. 3b). 194

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3.4 Relationships between soil fraction composition, C and N concentrations in soil particle-size fractions and bulk soil C and N storage

Across the transect, the concentrations and storage of C and N in bulk soils were negatively correlated with the content of sand and positively correlated with the contents of silt and clay (Fig. 4). The concentrations and storage of C and N in bulk soils were positively correlated with their concentrations in sand, silt, and clay (Fig. 5).

Stepwise multiple regression analyses allowed us to quantify the simultaneous effects of soil fraction composition, element concentrations in soil particle-size fractions, and BD on bulk soil C and N storage. The multiple regression model for C storage in bulk soils included the variables: clay C concentration (with the value of normalized regression coefficient for this variable = 0.70), clay content (0.45), silt content (0.21) and silt C concentration (-0.12), while sand content, sand C concentration and BD were excluded from the model. These variables together

accounted for 93.8% of the total variation of bulk soil C storage. In contrast, the 210 multiple regression model that best predicted soil N storage included: clay N 211 concentration (0.61), sand content (-0.30), clay content (0.27), BD (-0.10), and sand N 212 213 concentration (-0.05), while silt content and silt N concentration were excluded from the model. These variables accounted for 93.6% of the total variation of bulk soil N 214 storage. Inconsistent with the results of simple linear regressions, the stepwise 215 multiple regression analyses showed that C concentration in silt had a negative 216 correlation with soil C storage, and that sand N concentration had a negative 217 correlation with soil N storage. 218

220 4 Discussion

Across this 3000 km aridity gradient, sand was the most abundant fraction and 221 the contents of silt and clay were much lower, especially in soils from the extremely 222 arid sites. We suspect that this pattern is partly caused by the wind erosion and dust 223 224 storms which can be exacerbated by increasing aridity and frequently occur in higher 225 aridity areas of northern China (Wang et al., 2013; Yan et al., 2013; Zhang and Liu, 226 2010). Wind erosion favors losses of fine soil particles and consequently leads to changes of the soil texture (Feng et al., 2001; Wang et al., 2006; Yan et al., 2013). In 227 228 arid and semi-arid ecosystems experiencing increasing aridity, soils become more vulnerable to wind erosion because vegetation coverage declines (Zhang and Liu, 229 2010). Increased aridity is negatively correlated with aboveground net primary 230 231 productivity across this transect (Wang et al. 2014), which, could further decrease soil C and N storage through diminishing litter input (Yang et al., 2011). Similar to our 232 results, Liu et al. (2008) found that sand fractions in soils of steppe and meadow was 233 negatively correlated with MAP and positively correlated with MAT due to 234 drought-driven vegetation cover decline in the semi-arid East Asian steppe. 235

Other factors may also contribute to the pattern observed here. Parent materials, land use (e.g. grazing), and topography, can largely influence soil formation process and the contents of soil fractions (Barthold et al., 2013; Deng et al., 2015). For example, soils derived from limestone and quartzite have a lower content of sand fractions and a higher content of silt, compared to soils derived from granite (Belnap et al., 2014). In the grasslands of Inner Mongolia, climate and land use are of greater importance than parent material and topography in controlling soil type distribution
(Barthold et al., 2013). Therefore, we suspect that those factors associated with
climate would be more important than other factors in structuring the pattern of soil
particle-size distribution in our study sites.

246 Our results showed that C and N concentrations were highest in clay, followed by silt, and much lower in sand across a 3000 km aridity gradient. This pattern, which 247 may be caused because fine fractions in soil have high surface area which can 248 enhance formation of organo-mineral complexes that protect SOM from microbial 249 250 degradation (Hassink, 1997; Zhang and Liu, 2010), supports previous findings that soil fractionation is a useful tool for examining different C and N pools in soil 251 (Amelung et al., 1998; Gerzabek et al., 2001; Stemmer et al., 1999). Across the 252 253 transect, we observed that C and N concentrations and storage in bulk soils were negatively correlated with sand content and positively correlated with silt and clay 254 contents. Similarly, Bai et al. (2007) demonstrated that there was a negative 255 correlation between SOC content and sand content, and there were positive 256 correlations between SOC content and clay and silt contents based in wetland soils in 257 258 northeastern China. Positive correlations between soil C and N concentrations and silt and clay contents were also found in Inner Mongolian grasslands (He et al., 2014). 259

Supporting our first hypothesis, we found that C and N concentrations and storage in soil particle-size fractions tended to be negatively correlated with increasing aridity. The higher aridity sites have lower primary productivity (Wang et al., 2014) and thus a lower input of plant detritus into soil. Lower litter input is

correlated with lower C and N concentrations and storage in soil fractions (Yang et al., 264 2011; He et al., 2014). Paralleling our results, He et al. (2014) found that C and N 265 266 concentrations in soil particle-size fractions were positively correlated with MAP; moreover, they considered that MAP was better than MAT to model the variation of 267 268 soil C storage in an Inner Mongolia grassland. In the present study, we quantified the 269 relationship between soil C and N storage and aridity, which combines MAP and 270 MAT, across different sampling sites. Our results suggest that aridity is a robust predictor for the regional variation of C and N storage in soil fractions. 271

272 We found that C storage in sand was first decreased and then increased along the aridity gradient, which seems paradoxical given the results that the C concentrations 273 in sand linearly declined with increasing aridity while the content of sand linearly 274 275 increased with increasing aridity across the transect. The observed variation of C storage in sand across the transect may be due to the shifts of dominant controller for 276 the C storage in sand across the aridity transect. Sand C concentration appears to be 277 278 more important than sand content in driving the variation of sand C storage in the ecosystem with aridity value is less than 0.8 (where the C concentration in sand was 279 280 relatively higher and sand content was relatively lower). In contrast, sand content appears to be more important than C concentration in determining sand C storage 281 when the aridity value exceeds 0.8. Our results highlight the importance of looking at 282 both soil particle size and the C concentration of different particles in order to better 283 understand the influence of aridity on soil C pools. 284

285 We found that total C and N concentrations and storage in bulk soils generally

decreased with increasing aridity across the whole transect. Previous studies have 286 reported that soil C and N storage in the upper soil layers were positively correlated 287 288 with MAP and negatively correlated with MAT; these findings are similar to our 289 observations along a large aridity gradient (Follett et al., 2012; He et al., 2014; Liu et 290 al., 2012; Miller et al., 2004). The depletion of fine soil particles due to the intensified 291 wind erosion with increasing aridity could further deplete C and nutrients in arid systems because these particles have disproportionately greater amounts of C and 292 nutrients than larger particles (Yan et al., 2013). 293

294 Our results suggest that the decreases of bulk soil C and N storage along the aridity gradient resulted not only from the changes of composition of different soil 295 fractions but also from the decreases of C and N concentrations in each of those 296 297 fractions. While both soil C and N storage decreased with increasing aridity, the stepwise multiple regression analyses indicated that the simultaneous influences of 298 variation of different soil fractions and the element concentrations were different for 299 C and N. For bulk soil C storage, the most robust regression model did not include 300 sand content, sand C concentration, and BD, whereas for bulk soil N storage, silt 301 content and silt N concentration were excluded from the model. Our results thus 302 demonstrate that sand content is less important than silt content for controlling 303 variation of soil C storage, whereas silt is less important for the variation of soil N 304 storage at regional scale in the arid and semi-arid grasslands of northern China. 305

These findings are somewhat in agreement with previous findings that C is readily mineralized from un-complexed organic matter in sand-sized aggregates

whereas N is not, while silt tends to be more enriched in C than N (Christensen, 2001). 308 We found that clay content and clay element concentrations were the most important 309 310 factors for predicting the variation of both the soil C and N storage across this aridity gradient. Similarly, Burke et al. (1989) observed that clay was an important predictor 311 of soil C for American grassland soils. Together, these results indicate differences in 312 313 the relative importance of different soil particle-size fractions in driving soil C and N storage, although it is generally accepted that the dynamics of those two elements in 314 soils are closely correlated (Finzi et al., 2011). 315

316 This large-scale field investigation provides strong evidence that increasing aridity would reduce the soil C and N storage in arid and semi-arid ecosystems due 317 both to the changes of particle-sized fractions in soils (i.e. relatively more coarse 318 319 fraction content, but less fine fraction content with increasing aridity) and to the decline of C and N concentrations in each fraction. This study provides novel insights 320 into the patterns underlying regional changes of soil C and N from a soil particle-size 321 322 fractions perspective. Given the predicted increases in aridity in this century for the global drylands (Dai, 2013), this study indicates that the soil C and N pools in those 323 324 arid ecosystems may decline in the future. Because wind erosion would lead to greater loss of relatively fine silt and clay particles (Yan et al., 2013), our results 325 suggest that land use practices which reduce wind erosion (e.g. reducing the intensity 326 of grazing) will play an important role in sustaining soil C sequestration in dryland 327 328 regions globally.

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- 468 Figure legend
- 469 Fig. 1 Relationships of soil particle-size fractions contents with aridity. Data are presented as 470 mean \pm 1SE (n=5)
- 471
- 472 Fig. 2 Relationships of C and N concentrations in bulk soil and soil particle-size fractions with
- 473 aridity (a: C concentration; b: N concentration). Data are presented as mean \pm 1SE (n=5)
- 474
- 475 Fig. 3 Relationships of C and N storage in bulk soil and soil particle-size fractions with aridity (a:
- 476 C storage; b: N storage). Data are presented as mean \pm 1SE (n=5)
- 477

478 Fig. 4 Relationships of C and N concentrations and storage in bulk soil with the contents of soil

- 479 particle-size fractions in bulk soil (a: C concentration; b: C storage; c: N concentration; d: N
- 480 storage). Data are presented as mean ± 1 SE (n=5)
- 481
- 482 Fig. 5 The C and N concentrations and storage in bulk soil as affected by the concentrations of C
- 483 and N in soil particle-size fractions (a: C concentration; b: C storage; c: N concentration; d: N
- 484 storage). Data are presented as mean ± 1 SE (n=5)



489 Figure 2





492 Figure 3









