



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

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Abstract.

15 Climate factors such as aridity significantly influence soil carbon (C) and
nitrogen (N) stocks in terrestrial ecosystems. Further, soil composition plays an
important role in driving changes of soil C and N stocks at regional scale. However,
it remains uncertain whether such changes result from the variation of different soil
particle-size fractions or the C and N concentrations in those fractions. We examined
20 the distribution of total C and N in both bulk soil and different soil particle-size
fractions, including sand (53-2000 μm), silt (2-53 μm) and clay (<2 μm), along a
3000 km transect in arid and semi-arid grasslands of northern China. Across the
whole transect, sand content was positively and silt content was negatively
correlated with increasing aridity. Carbon stock in bulk soils (0-10 cm) ranged from
25 4.36 to 46.16 Mg C ha^{-1} , while N stock ranged from 0.22 to 4.28 Mg N ha^{-1} across
different sampling sites on the transect. The total C and N concentrations and stocks
in bulk soils as well as in the three particle-size fractions tended to be negatively
correlated with aridity. The concentrations and stocks of total C and N in bulk soils
were positively correlated with silt and clay contents and negatively correlated with
30 sand content. There were positive correlations between the concentrations and stocks
of C or N in bulk soils and the C or N concentrations in the three soil particle-size
fractions. By characterizing such a large scale aridity gradient, our results highlight
that aridity would decrease soil C and N stocks both by favoring increased sand
content and by decreasing C and N concentrations in all the three soil fractions.
35 These patterns thus have significant implications for understanding soil C and N



sequestration under scenarios of increasing aridity in global drylands that are predicted to occur this century.

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

40 1 Introduction

Grasslands, which cover nearly 40% of the world's land area, store approximately one-third of the total C in terrestrial ecosystems (He et al., 2012; White et al., 2000). Consequently, C turnover in grassland soils is considered to be a critical component of the global C cycle (Fisher et al., 1994; Wang et al., 2009). In
45 China, grasslands account for 41.7% of terrestrial land area and are mainly distributed in arid and 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, more extreme precipitation regimes, and global warming
50 variations due to variability in both climate and soil characteristics. Greater understanding of the controls on regional variation of soil C and N stocks in Chinese grasslands would facilitate projections of regional C and N cycling under global change scenarios.

Soil C and N stocks in grassland ecosystems are closely correlated with climatic
55 conditions. In arid and semi-arid ecosystems, soil C and N stocks are positively correlated with mean annual precipitation (MAP) at the regional scale (He et al., 2014; Nichols, 1984). This positive relationship is driven by the fact that water



availability is the dominant limiting factor for plant growth (and thus soil organic matter inputs) in these ecosystems. In contrast, mean annual temperature (MAT) is
60 negatively correlated with soil C and N stocks, 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
65 and Bertiller, 2010; Delgado-Baquerizo et al., 2013; Sanaullah et al., 2014).

Variation in soil particle-size fractions also exerts significant controls on the stock and turnover of soil organic matter (SOM) (Chen et al., 2010; Christensen, 2001; Qin et al., 2010) and increasing attention has focused upon the responses of C and N pools in different soil particle-size fractions to climate change (Amelung et al.,
70 1998; He et al., 2014). Clay and silt fractions in soil usually have higher C and N concentrations and stocks than that of sand fraction, so soils with higher clay and silt contents generally have higher soil organic C (SOC) and N stocks (Amelung et al., 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
75 particles; these 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 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; Chen and Chiu, 2003).



80 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 stocks in those regions (Delgado-Baquerizo et al., 2013; Sanaullah et al., 2014).
Variation in soil fraction composition and the C and N concentrations in different
85 soil particle sizes 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, compared with these well-described patterns of variation in soil C and N
stocks along climate and soil particle size gradients, the relative influence of these
factors on terrestrial C and N pools under climate change scenarios such as
90 increasing aridity is less clear (Delgado-Baquerizo et al., 2013).

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 the regional scale, we collected soil samples from 58 sites along a 3000 km
aridity transect across arid and semi-arid grasslands in northern China. The
95 distinctive features of this transect include a continuum of xeric to mesic grassland
types, ascending MAP and descending MAT from the west to the east, complete
meteorological records, relatively gentle geographical relief, and light human
disturbances (Luo et al., 2015). The objectives of this study were to: (1) examine the
distribution of C and N in various particle-size fractions of soils across the aridity
100 gradient, and (2) evaluate the relative contributions of soil fraction components and
their element concentrations to the changes of soil C and N stocks across the aridity



gradient. We hypothesized that: (1) concentrations and stocks of C and N in soil
particle-size fractions would be negatively correlated with increasing aridity; and (2)
soil C and N stocks would decline with increasing aridity due to both an increase in
105 the relative proportion of sand to silt and clay and a decrease of C and N
concentrations in the three soil fractions.



2 Materials and methods

2.1 Study site and soil sampling

110 This study was carried out along a 3000 km west-east transect of arid and
semi-arid grasslands across Xinjiang, Gansu, and Inner Mongolian in northern China.
The longitude of the transect ranged from 87 °22'E to 123 °23'E and the latitude
ranged 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.

115 The aridity (calculated as “1—precipitation / evapotranspiration”) of this transect
ranged 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
desert steppe, typical steppe, and meadow steppe from the west to east across the
transect, with the primary productivity ranging from <math><10 \text{ g m}^{-2} \text{ yr}^{-1}</math> in the desert
120 steppe to $>400 \text{ g m}^{-2} \text{ yr}^{-1}$ in the meadow steppe, and showing a negative relationship
with aridity (Wang et al. 2014). The species richness per square meter ranged from 0
(no plants) in the west of the transect to >30 in the east parts (Lü et al. unpublished
data). The soil types were arid, sandy, calcium-rich brown loess, and belonged to
Kastanozem soil group in the Food and Agriculture Organization (FAO)
125 classification system (Cheng et al., 2009).

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



130 center of the large plot). In each subplot, five soil samples (0-10 cm) were randomly
collected by a 3.0 cm diameter soil corer and then totally mixed them together as one
composite sample which was then sieved through a 2.0 mm sieve. All of the soil
samples were returned to the lab and then air dried for further analysis. Soil bulk
density (BD) was calculated as the ratio of dry soil mass per unit volume of the
135 sampling core and expressed as g cm^{-3} (Grossman and Reinsch, 2002).

2.2 Particle-size fractionation

Particle-size fractionation was completed by disrupting soil aggregates of bulk
soil samples using ultrasonic energy and separating the particle-size fractions by a
combination of wet sieving and continuous flow centrifugation (Chen and Chiu,
140 2003; He et al., 2009). Briefly, 40 g of sieved soil (< 2 mm) following removal of
visible debris was dispersed in 200 ml of deionized water using a probe-type
ultrasonic cell disrupter system (scientz-IIID) operating for 15 min in the continuous
mode at 361 W. We used a sieve to separate sand (particle size, 53-2000 μm) by
manual wet sieving method with deionized water. Particles which consisted of silt
145 (2-53 μm) and clay (< 2 μm) passing through the sieve during the wet sieving
process were collected. In order to separate silt 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 the silt fraction sinks to the bottom while the clay
fraction remains suspended. The silt fraction was then re-suspended in 200 ml
150 deionized water and re-centrifuged at 476 rpm; this procedure was repeated 5 times.
The clay fraction was obtained by transferring the suspensions into new centrifuge



bottles and centrifuging them at 4000 rpm for 30 minutes. All the fractions were dried at 50 °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
155 an automatic element analyzer (Vario MACRO cube, Elementar Analysensysteme GmbH).

2.3 Statistical analysis

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



3 Results

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

170 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
175 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.

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

180 Total C (Fig. 2a) and N concentrations (Fig. 2b) in the bulk soil significantly
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
transect, C and N concentrations in all of the three particle-size fractions were
negatively correlated with increasing aridity (Fig. 2). The total C and N
185 concentrations in the soil particle-size fractions varied greatly among the three soil
fractions, with the highest concentrations in clay (36.06 ± 1.49 g C kg⁻¹, 3.90 ± 0.17
g N kg⁻¹) and the lowest in sand (5.19 ± 0.56 g C kg⁻¹, 0.37 ± 0.04 g N kg⁻¹) (*p* <
0.001 in both cases).

190 3.3 C and N stocks in bulk soil and different soil particle-size fractions



Across the whole transect, C stock in bulk soils (0-10 cm) ranged from 4.36 to 46.16 Mg C ha⁻¹, while N stock ranged from 0.22 to 4.28 Mg N ha⁻¹. We found negative correlations between C and N stocks in each soil particle-size fraction and aridity, with the exception of C stocks in sand ($y=57.12-161.29x+114.70x^2$, $R^2=0.48$,
195 $p<0.0001$), which first decreased and then increased with increasing aridity (Fig. 3a). Paralleling this pattern, C stocks in bulk soils first decreased and then slightly increased with increasing aridity, with the lowest value presented in sites with aridity of ~ 0.8 ($y=160.49-371.88x+228.73x^2$, $R^2=0.73$, $p<0.0001$, Fig. 3a). Nitrogen stocks in bulk soils were negatively correlated with aridity (Fig. 3b).

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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 stocks

Across the transect, the concentrations and stocks of C and N in bulk soils were negatively correlated with the content of sand and positively correlated with the
205 contents of silt and clay (Fig. 4). The concentrations and stocks 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
210 fractions, and BD on bulk soil C and N stocks. The multiple regression model for C stocks 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
215 accounted for 93.8% of the total variation of bulk soil C stock. In contrast, the
multiple regression model that best predicted soil N stock included: clay N
concentration (0.61), sand content (-0.30), clay content (0.27), BD (-0.10), and sand
N 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
220 soil N stock. Inconsistent with the results of simple linear regressions, the stepwise
multiple regression analyses showed that C concentration in silt had a negative
correlation with soil C stock, and that sand N concentration had a negative
correlation with soil N stock.



225 4 Discussion

Across this 3000 km aridity gradient, sand was the most abundant fraction and the contents of silt and clay were much lower, especially in soils from the extremely arid sites. We suspect that this pattern is partly caused by the wind erosion and dust storms which can be exacerbated by increasing aridity and frequently occur in higher aridity areas of northern China (Wang et al., 2013; Yan et al., 2013; Zhang and Liu, 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 arid and semi-arid ecosystems experiencing increasing aridity, soils become more vulnerable to wind erosion because vegetation coverage declines (Zhang and Liu, 2010). Increased aridity is negatively correlated with aboveground net primary productivity across this transect (Wang et al. 2014), which, could further decrease soil C and N stocks through diminishing litter input (Yang et al., 2011). Similar to our results, Liu et al. (2008) found that sand fractions in soils of steppe and meadow was negatively correlated with MAP and positively correlated with MAT due to drought-driven vegetation cover decline in the semi-arid East Asian steppe.

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.

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 may be caused because fine fractions in soil have high surface area which can enhance formation of organo-mineral complexes that protect SOM from microbial 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 (Amelung et al., 1998; Gerzabek et al., 2001; Stemmer et al., 1999). Across the transect, we observed that C and N concentrations and stocks in bulk soils were negatively correlated with sand content and positively correlated with silt and clay contents. Similarly, Bai et al. (2007) demonstrated that there was a negative correlation between SOC content and sand content, and there were positive correlations between SOC content and clay and silt contents based in wetland soils in 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).

Supporting our first hypothesis, we found that C and N concentrations and stocks 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
270 correlated with lower C and N concentrations and stocks in soil fractions (Yang et al.,
2011; He et al., 2014). Paralleling our results, He et al. (2014) found that C and N
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
soil C stock in an Inner Mongolia grassland. In the present study, we quantified the
275 relationship between soil C and N stocks and aridity, which combines MAP and
MAT, across different sampling sites. Our results suggest that aridity is a robust
predictor for the regional variation of C and N stocks in soil fractions.

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



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

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

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,
315 2001). We found that clay content and clay element concentrations were the most
important factors for predicting the variation of both the soil C and N stocks across
this aridity gradient. Similarly, Burke et al. (1989) observed that clay was an
important predictor of soil C for American grassland soils. Together, these results
indicate differences in the relative importance of different soil particle-size fractions
320 in driving soil C and N stocks, although it is generally accepted that the dynamics of
those two elements in soils are closely correlated (Finzi et al., 2011).

This large-scale field investigation provides strong evidence that increasing
aridity would reduce the soil C and N stocks in arid and semi-arid ecosystems due
both to the changes of particle-sized fractions in soils (i.e. relatively more coarse
325 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 into the patterns underlying regional changes of soil C and N from a soil
particle-size 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
330 pools in those 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 suggest that land use practices which reduce wind erosion (e.g. reducing
the intensity of grazing) will play an important role in sustaining soil C sequestration
in dryland regions globally.



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475 Figure legend

Fig. 1 The relationships of soil particle-size fractions contents with aridity differed, with positive correlation between sand content and aridity, negative correlation between silt content and aridity, and no significant relationship between clay content and aridity. Data are presented as mean \pm 1SE (n=5)

480

Fig. 2 Carbon (a) and N concentrations (b) in all of the three particle-size fractions, as well as in bulk soils, were negatively correlated with increasing aridity. Data are presented as mean \pm 1SE (n=5)

485 **Fig. 3** The C (a) and N stocks (b) in bulk soil and soil particle-size fractions were generally negatively correlated with aridity. Data are presented as mean \pm 1SE (n=5)

Fig. 4 Across the transect, the concentrations and stocks of C (a, b) and N (c, d) in bulk soils were negatively correlated with the content of sand and positively correlated with the contents of
490 silt and clay. Data are presented as mean \pm 1SE (n=5)

Fig. 5 The concentrations and stocks of C (a, b) and N (c, d) in bulk soils were positively correlated with their concentrations in sand, silt, and clay. Data are presented as mean \pm 1SE (n=5)

495



Figure 1

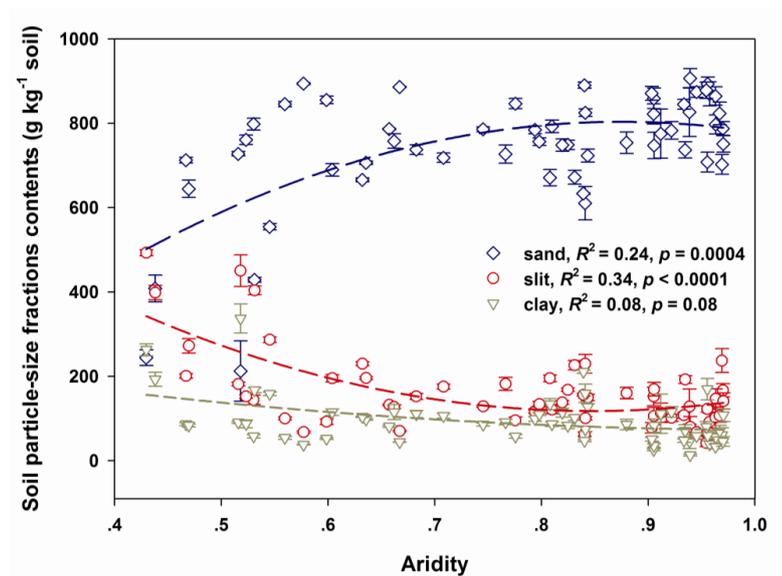
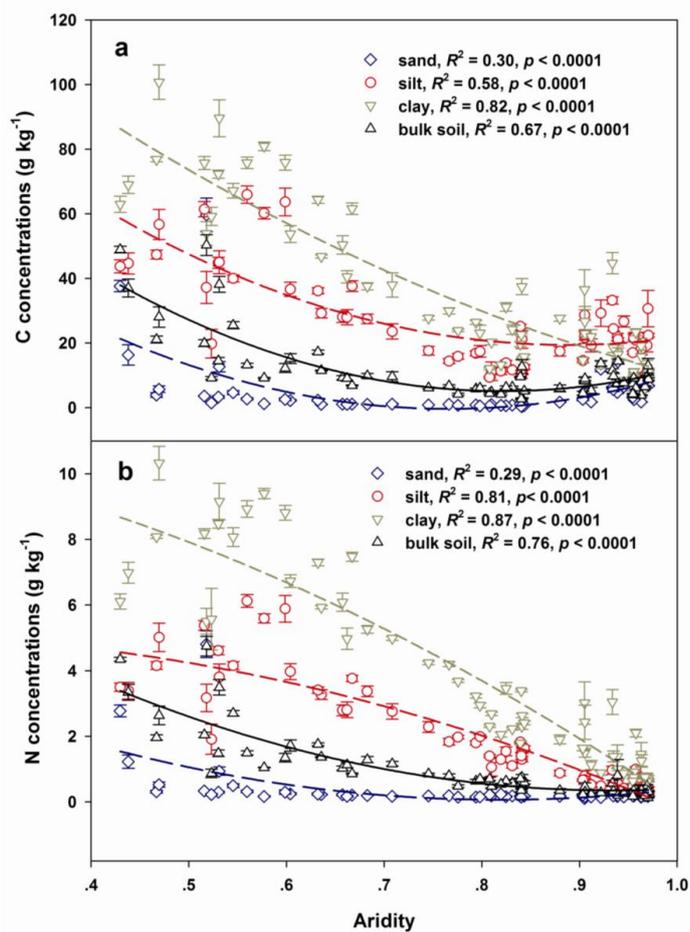




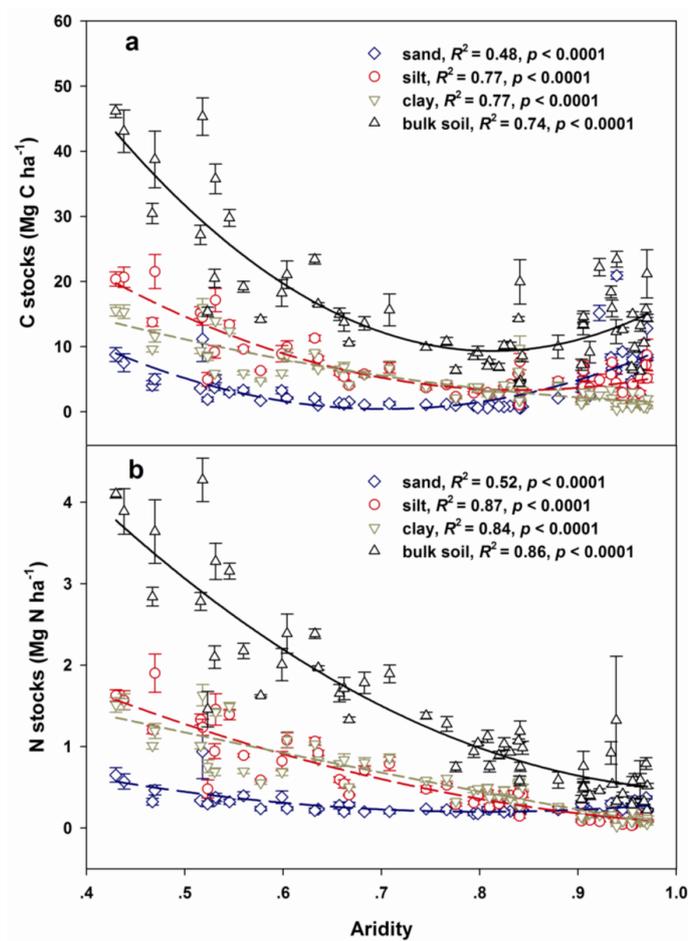
Figure 2



500



Figure 3





505 Figure 4

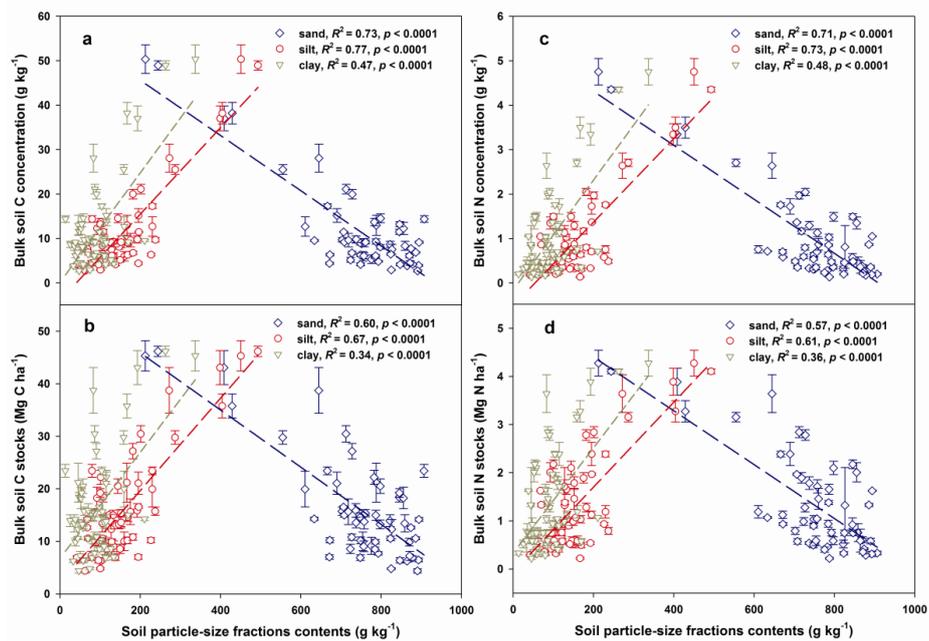




Figure 5

