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Distribution of soil nutrients under and outside tree/shrub canopies on a revegetated loessial slope

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\textbf{ABSTRACT}

Studies of soil nutrients in revegetated land have often not provided the sampling positions on a scale of individual trees/shrubs, suggesting that nutrients were assumed to not vary substantially at fine scales. This assumption, however, conflicts with the “fertile island” theory for arid and semi-arid areas. We assessed the importance of
sampling position on nutrient contents in 0-100 cm soil profiles by examining differences between soils under and outside the canopies of *Armeniaca sibirica* and *Caragana korshinskii* on a slope on the Loess Plateau, China. Soil organic carbon (OC), total nitrogen (TN), total phosphorus (TP), ammonium N (NH$_4^+$-N), and extractable P (EP) did not differ significantly under and outside the canopies, except for nitrate N (NO$_3^-$-N). The differences between these two canopy positions were significantly larger for *C. korshinskii* than *A. sibirica* for TP, significantly larger on upper than middle and lower slope sections for TN and NO$_3^-$-N. The NO$_3^-$-N content varied with sampling position around individual trees/shrubs, and trail tests about sampling position can be conducted around individual leguminous plants, in flatter areas, and in top-soil.

**Key words:** spatial variability, tree canopy, plant species, topography, soil profile

**Abbreviations:** OC- soil organic carbon; TN- soil total nitrogen; TP- soil total phosphorus; NO$_3^-$-N- soil nitrate nitrogen; NH$_4^+$-N- soil ammonium nitrogen; EP: soil extractable phosphorus.

**INTRODUCTION**

Revegetation of grassland is increasing rapidly in many parts of the world, and has attracted current scientific interest for its contribution to mitigating emissions of carbon dioxide, controlling soil erosion, restoring degraded land and potential impacts
on ecosystem function and long-term sustainability (Jackson et al. 2002; Makitalo et al. 2010; Don et al. 2011; Principe et al. 2015; Buendia et al. 2016). The transition from grassland to tree/shrub plantation modifies the quantity and quality of above and below ground biomasses, soil organic matter content, vegetation structure, microclimate conditions, and soil nutrient cycles (Céspedes-Payret et al. 2012; Souza et al. 2013). Altered pools and cycles of soil nutrients in turn can affect biomass production and ecosystem functioning. A clear description of the contents and availabilities of soil nutrients in revegetated areas is basic and essential for understanding the ecological consequences of this change in land use and may have important implications for long-term nutrient availability, sustainable productivity and future land management.

Many studies have examined the effects of transition from grassland to woodland on soil organic matter and nutrients at local (Chen et al. 2016), regional (Berthrong et al. 2012), and global (Don et al. 2011) scales. Our understanding of the influences of grassland revegetation on soil nutrients is mostly from analyses of soil samples. Estimates for large landscapes and ecosystems depend on extrapolation from localized data (Throop and Archer 2008). Most of these studies, however, did not provide detailed descriptions of the sampling positions around individual woody plants, such as under or outside the canopies. This omission suggests that soil nutrient contents were assumed to not differ under or outside canopies in revegetated areas.

The introduction of shrubs or trees to grassland increases the spatial heterogeneity of the soil (Throop and Archer 2008; Wei et al. 2013). Fine-scale spatial variations
around individual plants in arid and semi-arid areas often produce resource rich and poor patches under and between shrub canopies, respectively, creating fertile islands (Klemmedson and Barth 1975; De Boever et al. 2015; Waring et al. 2015). Several mechanisms contribute to this phenomenon, including increased litter inputs, root secretions, stem flow, animal excrement, and sedimentation of atmospheric particles (Rong et al. 2016); decreased erosion or increased deposition (Field et al. 2012); ameliorated microclimate with lower evaporation and temperature (Kidron 2009). Soil properties under individual plants can differ from those of neighbours by an average of 41% globally (Waring et al. 2015). The scarcity of data both under and outside shrub/tree canopies may thus limit estimation of soil nutrients in plantations. For example, Fernandez-Ondono et al. (2010) found a significant difference in organic carbon (OC) contents under the canopies of pine trees in plantations and in nearby open zones. Wei et al. (2010) reported that OC and total nitrogen (TN) contents and stocks were significantly greater under crowns than outside in Korshinsk pea shrub (Caragana korshinskii) plantations. These studies highlight the fine-scale variations of soil nutrients around individual shrubs/trees, which is critical information for optimal evaluations of revegetation effects.

Individual plant have pervasive and strong effects on soil chemistry, and these effects are stronger in deserts and tundra than forests (Waring et al. 2015). Dense canopies in forests may attenuate the differences of soil nutrient contents under or outside canopies of individual trees (Powers et al. 2004). Areas under canopies are not well defined, but are broad transitional areas that produce “zones of influence”
(Bedford and Small 2008). For example, De Boever et al. (2015) reported higher soil nutrient concentrations within an area up to 175% of the canopy radius. These studies suggest that neglecting sampling position around individual plants would influence measurements of soil properties in revegetated ecosystems.

In addition to the presence of plants, other biotic and abiotic factors have been associated with the effects of individual plants on soil properties, such as plant species (Li et al. 2007), stand age (De Boever et al. 2015), topography (Harman et al. 2014), and soil texture (Wei et al. 2010). For example, the effects of individual plants are different on hillslopes and in flat areas, because slopes alter ecological processes such as runoff, erosion, and sediment deposition, which in turn alter soil resources around individual plants (Harman et al. 2014). Li et al. (2007) found more pronounced fertile islands in *Tamarix* spp. than *Haloxylon ammodendron* Bge. due to their morphological differences in capturing and maintaining litter under canopies. These factors further increase the complexity of choosing sampling positions on an individual tree/shrub scale.

The Loess Plateau of China has undergone extensive vegetation restoration since the 1980s (Cheng et al. 2015), especially in the “Grain for Green” project initiated in 1999 (Chen et al. 2015), to address the severe losses of soil and water, and to improve the fragile ecosystem in this region. Remote sensing data show coverage of vegetation in this area has increased from 31.6% in 1999 to 59.6% in 2013 (Chen et al. 2015). Trees/shrubs have been planted to improve the potential for sequestering C, timber production, and economic benefits. Soil nutrient contents affected by revegetation in
former farmland or grassland have been well documented (e.g. Jin et al. 2016; Tong et al. 2016), but most studies have sampled soils without considering sampling positions around individual trees/shrubs, and little attention has been paid to the differences of nutrient concentrations sampled under and outside canopies. The main objective of this study was to investigate the effect of sampling position on the distribution of soil nutrients in revegetation land. Specifically, our objectives were to identify any differences of soil C, N, and P contents under and outside vegetation canopies, and determine the relationships between the differences with plant species, slope section, and soil depth.

**MATERIALS AND METHODS**

**Site description**

The study was conducted in the Liudaogou catchment, Shenmu County, Shaanxi Province, China (38°46′-38°51′N, 110°21′-110°23′ E; 1094-1274 m a.s.l.) (Figure 1a and 1b). Shenmu County has a moderate semiarid climate, with a mean annual air temperature of 8.4 °C and a mean annual precipitation of 437 mm, approximately 70% of which falls between June and September. The soil is classified as a Calcaric Regosol (the FAO-UNESCO classification system), developed from nutrient-poor loess. In the 0-10 cm layer OC is 4.39 g kg⁻¹, TN 0.41 g kg⁻¹, and total phosphorus (TP) 0.58 g kg⁻¹. The Liudaogou catchment is in the water-wind erosion transitional belt on the Loess Plateau and is subjected to both wind and water erosion. Most of the natural vegetation
was destroyed by long-term human activities, and the area has been revegetated mainly by dryland perennial plants, such as *Medicago sativa*, *C. korshinskii*, *Pinus tabuliformis* and *Armeniaca sibirica*.

**Field investigation and soil sampling**

The field investigation and collection of soil samples were conducted in September 2014 on a southeast-facing slope with loessial soil (9.0, 88.9, and 2.1% for particles <0.002, 0.002-0.2, and >0.2 mm, respectively). A tree species, *A. sibirica*, and a shrub species, *C. korshinskii*, were planted on the slope (Figure 1c) about 35 years ago according to local elders. *Caragana korshinskii*, a leguminous, sand-fixing, drought-resistant shrub, has been widely planted on the northern Loess Plateau since the 1980s. *Armeniaca sibirica* is indigenous to this region. A GPS receiver was used to record the geographical coordinates and elevations of the sampling sites, then distance between each sampling location was measured using BaseCamp 4.6.2 (Garmin Inc., 2016, Taiwan, China). Slope gradients were calculated from differences in distance and elevation between sampling locations. The slope is about 500 m long and from 1201 m a.s.l. at the bottom to 1257 m a.s.l. at the top. The herbaceous vegetation currently on the slope are primarily *Stipa bungeana*, *Astragalus melilotoides*, and *Melilotus albus*. Slope sections were defined as upper, middle, and lower, by dividing the slope into three equal parts from top to bottom. The effects of individual revegetated trees/shrubs, plant species, and slope section on nutrient contents were investigated. Pits (106 cm in diameter and 11 cm in depth) around the *A.*
*sibirica* boles remained from plantation preparation (Figure 2c), and the land was not visually disturbed in the *C. korshinskii* plantation (Figure 2d).

Six sampling locations were established and numbered as Ai (i=1, 2… 6) along a transect from the base of the slope upward (Figure 1c). A 20×20 m plot was established at each location. We then counted the number of trees/shrubs in each plot and measured their heights, diameters at breast height, and canopy areas. Three standard trees/shrubs were selected as replications at each location. The above ground herbaceous biomasses under and outside the canopies were collected from 1×1 m subplots, and the samples were oven-dried at 65 °C for 72 h to estimate dry weight. Detailed geographic and plant-growth information is shown in Table 1.

Soil samples were collected with a tube auger 5 cm in diameter under and outside the canopies (Figure 3) at six depth increments: 0-10, 10-20, 20-40, 40-60, 60-80, and 80-100 cm. The samples under the canopies were collected at a distance from the bases of half canopy radius (0.83±0.03 and 0.69±0.03 m for *A. sibirica* and *C. korshinskii*, respectively) because Throop and Archer (2008) reported that mid-canopy samples were the most accurate to represent soil chemical properties under canopies. Canopy size and coverage (means 42.83% for *A. sibirica* and 76.51% for *C. korshinskii*) for the two species differed at the study site, so we collected the samples outside the canopies at half a canopy radius beyond the drip line to avoid influences from neighboring trees/shrubs. Samples were collected from both the left and right sides of the trees/shrubs in the direction perpendicular to the slope aspect to avoid any differences in sampling between upslope and downslope (Bedford and Small 2008),
and these two samples were bulked.

A nearby area with *C. korshinskii* and aeolian sandy soil (1.6, 36.3, and 62.1% for particles <0.002, 0.002-0.2, and >0.2 mm, respectively) was selected to investigate whether the effect of canopy location on soil nutrient distribution was influenced by soil texture. The *C. korshinskii* in the aeolian soil was planted simultaneously with those in loessial soil and had similar conditions of plant growth: height (1.89±0.10 m), diameter at breast height (1.73±0.07 cm), and plant coverage (69.02%). Soils were sampled with the same procedure (Figure 3).

**Analysis of soil samples**

The samples were collected, air-dried, and passed through 2.0- and 0.25-mm sieves for laboratory analyses. The samples with particle sizes <2.0 mm were used for determining ammonium N (NH$_4^+$-N), nitrate N (NO$_3^-$-N) and extractable P (EP) contents, and samples with particle sizes <0.25 mm were used for determining OC, TN, and TP contents; OC, TN and EP contents was determined by Walkley-Black, Kjeldahl, and Olsen method, respectively (Page et al. 1982). TP content was quantified colorimetrically after digestion with HClO$_4$-H$_2$SO$_4$. Extractable N (NH$_4^+$-N and NO$_3^-$-N) was measured using a Lachat Flow Analyzer (AutoAnalyzer-AA3, Seal Analytical, Mequon, WI) after extraction by 2 mol L$^{-1}$ KCl (Kachurina et al. 2000).

We used relative differences to account for the differences of soil nutrient contents under (A) and outside canopies (B), where relative difference = (A-B)/B×100% (Don et al. 2011). Positive relative differences indicate that more soil
nutrients have accumulated under than outside trees/shrubs canopies.

**Statistical analyses**

All data were tested for normality before analysis. A four-way repeated-measures ANOVA was used to test the effects of canopy location (under and outside), plant species (*A. sibirica* and *C. korshinskii*), slope section (lower, middle, and upper), and soil depth (0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm layers), using the canopy location as a repeated factor (Von Ende 2001). We calculated the relative differences of nutrient contents between soils under and outside plant canopies. A two-way ANOVA was used to compare nutrient contents for canopy and soil depth in the nearby aeolian sandy soil. Paired *t*-test at a confidence interval of 95% were used to determine the significance of differences between under and outside soils. A simple linear correlation analysis was performed to determine if the relationships among the nutrients were altered under and outside the canopies.

A one-way ANOVA was used to compare the differences among different soil nutrient parameters. A three-way ANOVA was used subsequently to analyze the effects of plant species, slope section, and soil depth on the relative differences. In case of a significant main effect of slope section or soil depth, Duncan’s multiple range test was executed between all levels of that factor. Values were considered as significant different at *p*<0.05. All statistical tests were conducted in SPSS 16.0 for Windows (SPSS Inc., 2007, Chicago, USA).
RESULTS

Effect of canopy location on the distribution of soil nutrient contents

The relative differences of soil nutrient contents between under and outside tree/shrub canopies are shown in Figure 4. The relative difference was significantly higher for NO$_3^-$-N (16.77%) than the other five nutrients (4% on average). Only NO$_3^-$-N content was significantly improved under canopies compared with interspace areas ($p<0.05$), but OC, TN, TP, NH$_4^+$-N, and EP content were not significantly influenced by canopy location ($p>0.05$) (Table 2). Similar results were found in the neighboring aeolian sandy soil planted with C. korshinskii, where only NO$_3^-$-N was significantly influenced by canopy location ($p<0.05$) (Table 3). These results indicated that soil NO$_3^-$-N content but not OC, TN, TP, NH$_4^+$-N or EP contents were dependent on sampling position around individual trees/shrubs (under and outside canopies).

The correlations among C, N, and P contents under or outside the canopies are shown in Table 4. OC content under the canopies was positively correlated with TN, NO$_3^-$-N, and EP contents at $p<0.001$ but not significantly correlated with TP or NH$_4^+$-N content. Similar correlations were observed outside the canopies, but with lower correlation coefficients. Similar correlations among other soil nutrients were also observed under and outside the canopies, except among the NH$_4^+$-N, NO$_3^-$-N, and EP contents. These results indicated that collecting soil samples under or outside canopies did not affect the relationships among OC, TN, and TP contents, but altered
the relationships among extractable nutrients (NH$_4^+$-N, NO$_3^-$-N and EP).

**Effect of plant species on the relative differences of soil nutrient contents**

The effect of plant species on the relative differences of the nutrient contents under and outside the canopies was significant for OC, TN, and TP (Table 5). The relative differences for OC, TN, and TP contents were larger for *C. korshinskii* (9.34, 5.09, and 6.72%, respectively) than *A. sibirica* (1.20, 0.29, -0.71%, respectively) (Figure 5). TP content was significantly higher under (0.56 g kg$^{-1}$) than outside (0.53 g kg$^{-1}$) the canopies for *C. korshinskii* (*p*<0.05), but not significantly for *A. sibirica*, indicating larger effect of canopy location for *C. korshinskii* than *A. sibirica*. OC and TN contents did not differ significantly under and outside canopies for both *C. korshinskii* and *A. sibirica* (*p*>0.05), implying no significant effect of canopy location with species. These results indicated that the effect of canopy location on TP content was larger for *C. korshinskii* than *A. sibirica*.

**Effect of slope section on the relative differences of soil nutrient contents**

The effect of slope section on the nutrient relative differences under and outside tree/shrub canopies was significant for TN, TP, NO$_3^-$-N, and EP (Table 5), and this effect varied among the soil nutrients. The relative differences for TN and NO$_3^-$-N contents increased up the slope, and were largest in the upper slope section (8.50% for TN and 30% for NO$_3^-$-N) (Figure 6a and c), and canopy location had a significant effect in the upper section (*p*<0.05), indicating that the effect of canopy location was
larger in the upper slope section for TN and NO$_3^{-}$-N contents. The relative difference for TP content (10.57%) was largest in the middle slope section compared with the lower and upper sections, and canopy location only had a significant influence in the middle section rather than the lower or upper sections, indicating that the effect of canopy location was larger in the middle slope section for TP content. Canopy location had a significant effect on EP content in the lower and middle slope sections, and the relative difference for EP content (26.96%) was largest in the lower section. These results indicated that the effect of canopy location on N content was larger in the upper slope section, but had an inconsistent effect on P content.

**Effect of soil depth on the relative differences of soil nutrient contents**

Differences of OC, TN, and EP contents between under and outside canopies varied significantly with depth (Table 5). The relative differences were generally larger in the 0-40 cm layers, particularly the 10-20 cm layer for OC, TN, and EP contents (20.98, 13.31, and 21.82%, respectively) (Figure 7). The relative differences for OC, TN, and EP contents decreased to or below zero with increasing depth. Upon closer examination, however, OC, TN, and EP contents did not differ significantly between under and outside canopies in each soil layer ($p>0.05$). The differences between under and outside canopies for TP, NH$_4^{+}$-N, and NO$_3^{-}$-N contents were not significantly influenced by soil depth. These results indicated that the effect of canopy location was not influenced by soil depth, but tended to be larger for OC, TN, and EP contents in shallow layers (not significantly).
DISCUSSION

Influence of sampling position on soil nutrient measurements

Higher soil C, N, and P contents under than outside the canopies of the revegetated woody plants (Figure 4), support the “fertile island” perspective, as reported for other arid and semi-arid areas (Klemmedson and Barth 1975; Throop and Archer 2008; De Boever et al. 2015). This suggests that planting *A. sibirica* and *C. korshinskii* could improve C, N, and P contents on the Loess Plateau. Only NO$_3^-$-N content differed significantly between the canopy positions (Table 2), and the relationships among NO$_3^-$-N, NH$_4^+$-N, and EP contents differed between under and outside canopies (Table 4), because of the preferential enrichment of available nutrients in less fertile soils (Kellman 1979; Li et al. 2007). These results indicate that sampling position under or outside canopies should be considered when evaluating NO$_3^-$-N content. OC, TN, TP, NH$_4^+$-N, and EP contents, however, did not differ significantly between under and outside canopies (Table 2).

Waring et al. (2015) reported that plants would have a greater effect on soil chemistry in patchy landscapes than in ecosystems with continuous plant cover, because the effects for individual trees are smaller for closed canopies (Powers et al. 2004). Soil nutrient contents are higher under vegetation in wooded lands where the canopy coverage is usually low, between 20% and 30% (Vinton and Burke 1995; Bonanomi et al. 2008). Rodriguez et al. (2011) reported that the soil spatial variation...
was 17-fold higher under isolated individuals than inside the forest. Wei et al. (2010), also in Liudaogou catchment, showed that soil OC and TN concentrations and stocks in top 10 cm layer were nearly 2-fold higher under than outside canopies of *C. korshinskii*. Much smaller differences were for OC (6.31%) and TN (14.86%) between under and outside canopies at our study site. Coverage in the study by Wei et al. (2010) was <30% but averaged 76.51% at our study site (Table 1). Caldwell et al. (2012) reported that the biotic influence of shrubs extended about half a canopy radius beyond the drip line. Harman et al. (2014) found that the canopy could influence soil nutrient content 2-4 canopy radii outside the canopies, not just under the canopy. The lack of significant differences between the two microhabitats of individual trees/shrubs in our study may therefore be attributed to the high coverage of vegetation.

Herbaceous vegetation is not only an important source of organic matter but can also reduce soil erosion. Field et al. (2012) reported that self-reinforcing “fertile island” was due to the redistribution of material from increased erosion bare patches into shrub patches when herbaceous cover was reduced. Previous studies reporting higher soil nutrient contents under vegetation also reported more herbaceous biomass under than outside the canopies (Klemmedson and Barth 1975; Schade et al. 2003; Cao et al. 2016). Herbaceous above ground biomass at our study site was lower under than outside the canopies (Table 1). The flourishing herbaceous vegetation favoured the nutrient status in areas between trees/shrubs, which may partly account for the nonsignificant differences of nutrient contents under and outside the canopies.
Many of the studies reporting an enrichment zone under vegetation were conducted in sandy soils (e.g. Klemmedson and Barth 1975; Cao et al. 2016; Rong et al. 2016), and some reported that the influence of woody plants on soil nutrient contents was greater in sandy than fine-textured soils (Campbell et al. 1994; Rodriguez et al. 2009; Wei et al. 2013). The nonsandy texture of the soil in our study may have contributed to the lack of significant differences between nutrient contents under and outside the canopies. The results for the aeolian sandy soil, where NO$_3^-$-N content but not OC, TN, TP, NH$_4^+$-N or EP content was significantly affected by canopy location, were similar to the loessial soil (Table 3). The soil texture at our study site was thus not responsible for the lack of significant differences between under and outside canopies.

Effects of plant species, slope section and soil depth on nutrient content variations

The effect of canopy location on TP contents being more pronounced for *C. korshinskii* than *A. sibirica* (*p*<0.05), and OC and TN contents (although not significantly), have three potential reasons. First, *C. korshinskii* grows closer to the soil surface (Figure 2b and 2d) and can greatly reduce soil erosion and can intercept dust and sediments from wind and water erosion, which would increase nutrient contents under the canopies and lead to larger differences under and outside the canopies. Second, *C. korshinskii* is a legume which promotes accumulation of soil N and other nutrients, consistent with previous studies (Wei et al. 2010; Rodriguez et al. 2009; Wei et al. 2013).
Third, *A. sibirica* had less understory biomass (Table 1), and the soil near the stems was bare (Figure 2c). Soil nutrients could thus be redistributed from under canopies and intercepted by nearby grass (Turnbull et al., 2011). Plant morphology, N-fixing trait and understory biomass could therefore account for the larger difference between under and outside canopies for *C. korshinskii* than *A. sibirica*. The effects of *A. sibirica* and *C. korshinskii* on the properties of the surrounding soil were likely associated with the different canopy coverages (means 42.83 and 76.51%, respectively), but the properties did not differ significantly between the two canopy positions. The interconnected factors of plant species and canopy coverage impeded our understanding of the effect of each factor, so we recommend investigating the effect of plant species with similar canopy coverages.

The effect of woody plants on soil nutrients in slopes differs between slopes and flat areas. The effect of individual trees/shrubs on soil properties are more conspicuous in flatter areas than on steep hills, because plant effects can be dampened by environmental factors on steep slopes (Bedford and Small 2008; Harman et al. 2014; Hoogendoorn et al. 2016). Consistent with these studies, the differences of soil TN and NO$_3^-$-N contents in our study increased significantly with decreasing slope gradient (3.3, 7.8, and 14.6° in the upper, middle, and lower sections, respectively) (Figure 6a and c). Schade and Hobbie (2005) reported the positive effect of canopies on organic matter content was higher in an upland area than on a terrace. Consistent with that study, the differences of TN and NO$_3^-$-N contents in our study were higher in upper than lower slope sections. The difference of herbivory biomasses under and
outside the canopies increased up the slope (Table 1), and led to a larger difference of inputs of organic matter and nutrient cycling in the upper sections. The differences of P content in the three slope sections did not have a clear pattern (Figure 6b and d), partly because P has a low availability in calcareous soil (Halajnia et al. 2009). TP and EP contents were thus more affected by a biotic factor, plant species ($F=21.3$ and $35.3$, respectively), than by an abiotic factor, slope section ($F=18.0$ and $8.2$, respectively), especially for EP content (Table 2).

Klemmedson and Barth (1975) first established the vertical structure of fertile islands, and many studies have since confirmed that the influence of individual plants decreased with soil depth with fewer organic matter inputs and soil microorganisms (Miura et al. 2008, Rong et al. 2016). The differences of soil nutrients in our study, however, were not significantly affected by soil depth. This finding was largely attributed to the nonsignificant effects of canopy location on soil OC, TN, TP, $\text{NH}_4^+$-N, and EP content. The effect of canopy location on $\text{NO}_3^-$-N content did not significantly interact with soil depth. The differences of OC, TN, and EP contents in our study decreased with depth, although not significantly. For instance, the relative differences of OC and EP in our study decreased from 16.90 and 14.68% in 20-40 cm layer to -7.27 and -15.10% in 40-60 cm layer, respectively. Purbopuspito and Rees (2002) measured distribution of clove roots at 0.5, 1.0, and 1.5 canopy radii in a 0-100 cm soil profile and found that differences of root densities at these distances decreased with depth, especially below 37.5 cm. The differences of OC and EP contents in our study decreased at a depth of 40 cm, and the differences of TN content decreased at a
depth of 20 cm (Figure 7), similar to the finding by Purbopuspito and Rees (2002). The effect of woody plants have been reported to be highest in surface horizons, usually 0-5 or 0-10 cm layer (Klemmedson and Barth 1975; De Boever et al. 2015), but the differences in our study were larger in the 10-20 than 0-10 cm layer, partly because the severe erosion at our study site, which is in the crisscross zone of wind and water erosion, attenuated the effect of the trees/shrubs on the surface soil. Cheng et al. 2013 found that the fine root density (also the error bars) for *C. korshinskii* was higher in the 10-20 cm depth than the 0-10 cm depth in silty loam soil in the same study area, the Liudaogou watershed. A similar finding for *A. sibirica* was reported by Zhao et al. 2000. These findings suggest that high fine root density and its high spatial heterogeneity in the 10-20 cm soil layer contribute to the higher spatial heterogeneity of soil nutrients.

**CONCLUSIONS**

Our study reported four major findings about differences in soil nutrient (OC, TN, TP, NH$_4^+$-N, NO$_3^-$-N, and EP) contents under and outside tree/shrub canopies associated with plant species, slope section, and soil depth in a revegetated loessial slope. Only NO$_3^-$-N content, and not OC, TN, TP, NH$_4^+$-N, or EP contents, differed significantly between canopy position. The differences for TP contents between canopy position were larger for *C. korshinskii* than *A. sibirica*. The differences were larger in the upper slope section for TN and NO$_3^-$-N contents. The differences were
not significantly influenced by soil depth, and tended to be larger in the 10-20 cm layer for OC, TN, and EP contents in the 0-100 cm profile.

This study suggested that sampling position on a scale of individual tree/shrub is important when analyzing NO$_3^-$-N contents after revegetation. Trial samples can be tested when selecting sampling positions for leguminous plants, flatter areas, and shallow soil depths. These results can help to optimize sampling strategies in revegetated land on the Loess Plateau and similar areas around the world. Studies that systematically analyze the effect of canopy coverage on differences in soil properties under and outside canopies are needed.

ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (41390463, 41530854 and 41571221) and the Open Research Fund of the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (A314021402-1504). The authors acknowledge Xiaoxu Jia and Chunlei Zhao for their helpful advice on the manuscript, William Blackhall and Kunxuan Wang for their help with the language before submission, and Xuezhang Li, Bingxia Liu, and Sheng Wang for their assistance with the field experiment.

REFERENCES


Figure caption

Figure 1 (a) Location of the study site in China, (b) the Liudaogou catchment, and (c) diagram of the sampling locations along the transect on the slope. The maps were created using ArcGIS by Esri (Environmental Systems Resource Institute, ArcGIS 10.0; www.esri.com).

Figure 2 General view of (a) *A. sibirica* and (b) *C. korshinskii* on the upper slope, and the views around the bases of (c) *A. sibirica* and (d) *C. korshinskii* on 4 September 2014.

Figure 3 A diagram of the sampling positions under and outside the canopy of an individual plant.

Figure 4 Relative differences (mean ± SE) of soil OC, TN, TP, \( \text{NH}_4^+ \), \( \text{NO}_3^- \), and EP contents in the 0-100 cm soil layer. Different letters above the bars indicate significant differences for the various soil nutrients.

Figure 5 Relative difference (mean ± SE) of soil (a) OC, (b) TN, and (c) TP contents for *A. sibirica* and *C. korshinskii* in the 0-100 cm soil layer. Different letters above the bars indicate significant differences between the two plant species. Differences of soil nutrient content between under and outside canopies are denoted by * (p<0.05) and NS (p>0.05).

Figure 6 Relative differences (mean ± SE) of soil (a) TN, (b) TP, (c) \( \text{NO}_3^- \), and (d) EP contents for the lower, middle and upper slopes in the 0-100 cm soil layer. Different letters above the bars indicate significant differences among slope sections. Differences of soil nutrient content between under and outside canopies are denoted by * (p<0.05), ** (p<0.01), and NS (p>0.05).

Figure 7 Relative differences (mean ± SE) of soil (a) OC, (b) TN, and (c) EP contents in the 0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm layers. Different letters above the bars indicate significant differences among the soil layers. Soil nutrient contents did not differ significantly between under and outside canopies at each depth (p>0.05).
### Table 1 Geographic, soil, and vegetation characteristics of the study site.

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Slope section</th>
<th>Slope gradient (°)</th>
<th>Plant species</th>
<th>Status of growth</th>
<th>Coverage (%)</th>
<th>Herbaceous aboveground biomass (g m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>H (m)</td>
<td>DBH (cm)</td>
<td>Under canopy</td>
</tr>
<tr>
<td>A1</td>
<td>Lower</td>
<td>14.6</td>
<td><em>A. sibirica</em></td>
<td>2.84±0.25</td>
<td>6.01±0.81</td>
<td>64.45±14.57b</td>
</tr>
<tr>
<td>A2</td>
<td></td>
<td></td>
<td><em>C. korshinskii</em></td>
<td>1.72±0.07</td>
<td>1.46±0.12</td>
<td>202.11±25.88a</td>
</tr>
<tr>
<td>A3</td>
<td>Middle</td>
<td>7.8</td>
<td><em>C. korshinskii</em></td>
<td>1.78±0.21</td>
<td>1.69±0.19</td>
<td>148.48±18.29a</td>
</tr>
<tr>
<td>A4</td>
<td></td>
<td></td>
<td><em>A. sibirica</em></td>
<td>3.27±0.24</td>
<td>6.83±0.75</td>
<td>97.96±19.1b</td>
</tr>
<tr>
<td>A5</td>
<td>Upper</td>
<td>3.3</td>
<td><em>A. sibirica</em></td>
<td>2.98±0.37</td>
<td>7.46±1.43</td>
<td>67.16±12.77b</td>
</tr>
<tr>
<td>A6</td>
<td></td>
<td></td>
<td><em>C. korshinskii</em></td>
<td>1.98±0.17</td>
<td>1.58±0.11</td>
<td>239.76±23.95a</td>
</tr>
</tbody>
</table>

Note: H, height; DBH, diameter at breast height (*A. sibirica* was measured at a height of 1.35 m and *C. korshinskii* was measured at ground level). Different letters within a row indicate significant differences at \(p<0.05\).
Table 2  F-statistics and levels of significance from a four-way repeated-measures ANOVA showing the effect of plant species (P), slope section (S), soil depth (D), canopy (C) (repeated factor), and their interactions on soil OC, TN, TP, NH₄⁺-N, NO₃⁻-N, and EP contents.

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>OC</th>
<th>TN</th>
<th>TP</th>
<th>NH₄⁺-N</th>
<th>NO₃⁻-N</th>
<th>EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between-subject effect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>13.8***</td>
<td>6.5*</td>
<td>21.3***</td>
<td>12.4**</td>
<td>35.2***</td>
<td>35.3***</td>
</tr>
<tr>
<td>S</td>
<td>2</td>
<td>6.5**</td>
<td>2.2</td>
<td>18***</td>
<td>12.4***</td>
<td>10.5***</td>
<td>8.2**</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>143.6***</td>
<td>202.6***</td>
<td>0.9</td>
<td>0.9</td>
<td>93.3***</td>
<td>11.4***</td>
</tr>
<tr>
<td>P×S</td>
<td>2</td>
<td>34.5***</td>
<td>75.1***</td>
<td>51.7***</td>
<td>7.5**</td>
<td>7.3**</td>
<td>4.3*</td>
</tr>
<tr>
<td>S×D</td>
<td>10</td>
<td>3.1**</td>
<td>5.4***</td>
<td>1.1</td>
<td>0.6</td>
<td>1.2</td>
<td>1.3</td>
</tr>
<tr>
<td>P×D</td>
<td>5</td>
<td>0.8</td>
<td>1.9</td>
<td>0.1</td>
<td>0.2</td>
<td>25***</td>
<td>1.4</td>
</tr>
<tr>
<td>P×S×D</td>
<td>10</td>
<td>0.5</td>
<td>0.9</td>
<td>1.2</td>
<td>0.1</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Error (C)</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Within-subject effect                      |    |       |       |       |        |        |       |
| C            | 1  | 0.7    | 0.5   | 2.3   | 0.0   | 5.5*   | 0.9    |
| C×P          | 1  | 2.0    | 4.2*  | 5*    | 0.1   | 0.1    | 1.0    |
| C×S          | 2  | 2.4    | 4.8*  | 5.7** | 0.2   | 0.8    | 8.6*** |
| C×D          | 5  | 0.9    | 0.9   | 0.5   | 0.7   | 0.8    | 0.9    |
| C×P×S        | 2  | 2.7    | 0.4   | 3.6*  | 1.4   | 0.3    | 0.5    |
| C×S×D        | 10 | 0.5    | 0.3   | 0.3   | 0.7   | 0.4    | 1.4    |
| C×P×D        | 5  | 0.4    | 0.9   | 0.3   | 0.5   | 1.8    | 3.3**  |
| C×P×S×D      | 10 | 1.1    | 0.5   | 0.3   | 0.6   | 0.3    | 1.9    |
| Error (C)    | 72 |         |       |       |        |        |       |

Note: *p<0.05, **p<0.01, ***p<0.001

Table 3  F-statistics and levels of significance from a two-way ANOVA showing the effect of canopy (C), soil depth (D), and their interaction on soil OC, TN, TP, NH$_4^+$-N, NO$_3^-$-N, and EP contents for *C.korshinskii* in aeolian sandy soil.

<table>
<thead>
<tr>
<th></th>
<th>OC</th>
<th>TN</th>
<th>TP</th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
<th>EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>0.037</td>
<td>4.119</td>
<td>0.024</td>
<td>0.01</td>
<td>9.893**</td>
<td>1.081</td>
</tr>
<tr>
<td>D</td>
<td>5.736**</td>
<td>14.941***</td>
<td>1.28</td>
<td>1.262</td>
<td>16.536***</td>
<td>11.264***</td>
</tr>
<tr>
<td>C×D</td>
<td>1.756</td>
<td>1.619</td>
<td>0.974</td>
<td>0.36</td>
<td>5.273**</td>
<td>0.542</td>
</tr>
</tbody>
</table>

Note: *p*<0.05, **p*<0.01, ***p*<0.001

OC; soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; NO$_3^-$-N: soil nitrate nitrogen; NH$_4^+$-N: soil ammonium nitrogen; EP: soil extractable phosphorus.
**Table 4** Pearson correlation analysis of soil OC, TN, TP, NH$_4^+$-N, NO$_3^-$-N, and EP contents.

<table>
<thead>
<tr>
<th></th>
<th>TN</th>
<th>TP</th>
<th>NH$_4^+$-N</th>
<th>NO$_3^-$-N</th>
<th>EP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Under canopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>0.967***</td>
<td>-0.038</td>
<td>0.137</td>
<td>0.682***</td>
<td>0.524***</td>
</tr>
<tr>
<td>TN</td>
<td>-0.144</td>
<td>0.135</td>
<td>0.727***</td>
<td>0.455***</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td>0.179</td>
<td>0.002</td>
<td>0.119</td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td></td>
<td></td>
<td>0.183</td>
<td>0.123</td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$-N</td>
<td></td>
<td></td>
<td></td>
<td>0.258**</td>
<td></td>
</tr>
<tr>
<td><strong>Outside canopy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC</td>
<td>0.950***</td>
<td>-0.094</td>
<td>0.138</td>
<td>0.589***</td>
<td>0.508***</td>
</tr>
<tr>
<td>TN</td>
<td>-0.143</td>
<td>0.162</td>
<td>0.607***</td>
<td>0.501***</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td></td>
<td>0.083</td>
<td>-0.147</td>
<td>0.074</td>
<td></td>
</tr>
<tr>
<td>NH$_4^+$-N</td>
<td></td>
<td></td>
<td>0.251**</td>
<td>0.230*</td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$-N</td>
<td></td>
<td></td>
<td></td>
<td>0.171</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** *p<0.05, **p<0.01, ***p<0.001*

OC; soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; NO$_3^-$-N: soil nitrate nitrogen; NH$_4^+$-N: soil ammonium nitrogen; EP: soil extractable phosphorus.
Table 5 $F$-statistics and levels of significance from a three-way ANOVA showing the effect of plant species (P), slope section (S), soil depth (D), and their interactions on the relative differences of soil OC, TN, TP, NH$_4^+$-N, NO$_3^-$-N, and EP contents.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>R of OC</th>
<th>R of TN</th>
<th>R of TP</th>
<th>R of NH$_4^+$-N</th>
<th>R of NO$_3^-$-N</th>
<th>R of EP</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>1</td>
<td>4.2*</td>
<td>2.7*</td>
<td>13.6***</td>
<td>0.6</td>
<td>2.9</td>
<td>3.6</td>
</tr>
<tr>
<td>S</td>
<td>2</td>
<td>1.8</td>
<td>11.8***</td>
<td>16.7***</td>
<td>0.3</td>
<td>5.8*</td>
<td>14.1***</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>5.9***</td>
<td>2.6*</td>
<td>1.0</td>
<td>1.0</td>
<td>0.3</td>
<td>2.9***</td>
</tr>
<tr>
<td>P×S</td>
<td>2</td>
<td>8.5***</td>
<td>0.1</td>
<td>12.1***</td>
<td>3.0</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>S×D</td>
<td>5</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8</td>
<td>1.4</td>
<td>3.0</td>
<td>8.1***</td>
</tr>
<tr>
<td>P×D</td>
<td>10</td>
<td>0.4</td>
<td>0.8</td>
<td>0.6</td>
<td>1.3</td>
<td>2.2</td>
<td>3.1**</td>
</tr>
<tr>
<td>P×S×D</td>
<td>10</td>
<td>3.0</td>
<td>1.7*</td>
<td>0.7</td>
<td>1.4</td>
<td>2.3</td>
<td>4.4***</td>
</tr>
</tbody>
</table>

Note: *$p<0.05$, **$p<0.01$, ***$p<0.001$

R: relative difference = (under-outside)/outside×100%

OC; soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; NO$_3^-$-N: soil nitrate nitrogen; NH$_4^+$-N: soil ammonium nitrogen; EP: soil extractable phosphorus.
Figure 1 (a) Location of the study site in China, (b) the Liudaogou catchment, and (c) diagram of the sampling locations along the transect on the slope. The maps were created using ArcGIS by Esri (Environmental Systems Resource Institute, ArcGIS 10.0; www.esri.com).
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481x270mm (72 x 72 DPI)
Figure 3 A diagram of the sampling positions under and outside the canopy of an individual plant.

24x16mm (300 x 300 DPI)
Figure 4 Relative differences (mean ± SE) of soil OC, TN, TP, NH$_4^+$-N, NO$_3^-$-N and EP contents in the 0-100 cm soil layer. Different letters above the bars indicate significant differences for the various soil nutrients.

Relative difference = (under-outside)/outside × 100%

OC; soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus; NO$_3^-$-N: soil nitrate nitrogen; NH$_4^+$-N: soil ammonium nitrogen; EP: soil extractable phosphorus.
Figure 5: Relative difference (mean ± SE) of soil (a) OC, (b) TN, and (c) TP contents for A. sibirica and C. korshinskii in the 0-100 cm soil layer. Different letters above the bars indicate significant differences between the two plant species. Differences of soil nutrient content between under and outside canopies are denoted by * (p<0.05) and NS (p>0.05).

Relative difference = (under-outside)/outside×100%

OC: soil organic carbon; TN: soil total nitrogen; TP: soil total phosphorus.

93x31mm (300 x 300 DPI)
Figure 6 Relative differences (mean ± SE) of soil (a) TN, (b) TP, (c) NO3--N, and (d) EP contents for the lower, middle and upper slopes in the 0-100 cm soil layer. Different letters above the bars indicate significant differences among slope sections. Differences of soil nutrient content between under and outside canopies are denoted by * (p<0.05), ** (p<0.01), and NS (p>0.05).

Relative difference = (under-outside)/outside×100%

TN: soil total nitrogen; TP: soil total phosphorus; NO3--N: soil nitrate nitrogen; EP: soil extractable phosphorus.

179x173mm (300 x 300 DPI)
Figure 7 Relative differences (mean ± SE) of soil (a) OC, (b) TN, and (c) EP contents in the 0-10, 10-20, 20-40, 40-60, 60-80 and 80-100 cm layers. Different letters above the bars indicate significant differences among the soil layers. Soil nutrient contents did not differ significantly between under and outside canopies at each depth (p>0.05).

Relative difference = (under-outside)/outside×100%