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Abstract

Globally, grassland soils, if managed properly, are important for the sake of carbon sequestration. The quality and quantity of soil organic carbon is probably influenced by vegetation traits such as composition and plant species. We determined the influence of vegetation composition and co-dominant species on soil organic carbon in two *Prangus uloptera* stands. The stands situated at close proximity were identified in a *Prangos uloptera* community in north-western Iran. Both stands shared similar dominant plant species with the same physiographical and climatological characteristics, but differed in their co-dominant plant species. To compare soil organic matter (SOM) parameters, soil samples from each stand were collected from depths of 0-15cm and 15-30cm. The results showed that SOM had spatial variation which was probably affected by the plant species. The highest values of total C, Total N, POM-C, POM-N, microaggregates, C in macroaggregates and C in microaggregates in the upper soil layer occurred in the stand with the highest cover of *Astragalus microcephalus* and *Acanthophyllum microcephalum*. It is suggested that, in conservation projects based on higher carbon sequestration, the plant species with more incorporation of carbon content into soil should be identified and used more widely. .

Key words: Carbon sequestration, Plant species, Soil organic carbon, Vegetation type, *Prangus uloptera* stands.

1. Introduction

Soil organic matter (SOM), a key component of soil-plant ecosystems, is closely associated with soil features and processes (Chen et al., 2004). SOM can be divided into labile, slow and recalcitrant organic matter according to its turnover rate (Six et al., 2002). Labile SOM, with a shorter turnover time, can respond sensitively to changes in vegetation compared to total SOM in world ecosystems (Laik et al., 2009). However, previous studies showed that vegetation composition is likely to influence some soil parameters. For instance, Wardle and Van der Putten (2002) and Wardle and Zackrisson (2005) claimed that plant species could affect the number and activity of soil biota. In addition, the quality and quantity of SOM might depend on the nature and composition of the plant community. The quality and quantity of SOC (soil organic carbon) are also affected by vegetation traits such as composition and plant species. Studies investigating the effects of environmental conditions on soil ecosystems have mainly concerned forest ecosystems (e.g. Ashagrie et al., 2007; Christenson et al., 2009; Wang and Wang, 2011), shrubland (e.g. An et al., 2010), cultivated stands (e.g. Gartzia-Bengoetxea et al., 2009), crop production (e.g., Haynes, 1999; Huang et al., 2010; Beedy et al., 2010), land use (e.g. Bettina et al., 2005; Leifeld and Kögel, 2005) and overgrazed pastures (Fallahzade and Hajabbasi, 2011), but none of these studies focused on the effect of co-dominant species on total and labile SOM. In addition, the physical location (e.g. aggregates) of the organic matter in soil and how this will be influenced by vegetation composition in a single habitat is not well understood.

Rangeland ecosystems are very diverse (i.e. grassland, shrubland, savannas, hot and cold deserts, and tundra), and play an important role in storing carbon both above and below ground. In 1993, carbon (C) stored in the world's rangeland soils was estimated to be 591.6 Gt, or 44% of the world's total soil carbon (Solomon et al., 1993). Grassland soils are known for their high levels of organic matter and high structural stability (van Veen and Paul, 1981),

although they vary among habitats and vegetation types (Solomon et al., 1993). Preindustrial levels of soil C (to depths of 20 cm) in grassland alone have been estimated to be approximately 96 Gt (Ojima et al., 1993b). Soil C stocks for these grasslands are around 81-164 Gt, based on land cover estimates of Bailey (1989). As a result, grassland soils, if managed properly, are important for carbon sequestration and a small increase in SOC may decrease the atmospheric CO_2 concentration and alter the global climate positively (Bu et al., 2012).

The objective of this study was to determine the influence of vegetation composition and especially that of co-dominant species, on total C, total N, particulate organic matter C (POM-C), particulate organic matter N (POM-N), macro- and micro aggregate distribution and C-associated with macro- and micro-aggregates in a *Prangus uloptera*-dominated Iranian grassland.

2. Materials and Methods

2.1. Description of the study area

This study was carried out in north-western Iran, within the grassland ecosystem of the Khanghah watershed, extending over about 2000 ha between latitudes 37º46´18"N and 37º50´42"N and longitudes 44º57´04"E and 45º00´32"E. Mean annual precipitation, temperature and altitude are 393.9 mm, 9.87 ºC and ca. 1725 m a.s.l., respectively. *Prangos uloptera* DC plant communities were selected for the study. The *Prangus* genus is widespread in north-western Iran and consists of 15 species. *Prangos uloptera* is one of the most important species and has medicinal, industrial and foraging uses. The community was distinguished according to the dominant plant species (Heady and Child, 1994) (here *Prangos uloptera*), and two adjacent stands were identified in the community. Both stands were classified as "good" for rangeland condition and "stable" for rangeland trend according to

Holechek's (1989) method (Motamedi et al. 2009). In both stands, the percentage cover of dominant plant species and other physiographical and climatological characteristics were similar and the stands mostly differed in the co-dominant plant species. In the first stand (hereafter coded *Prangos uloptera1*), the co-dominant plant species were *Pterpyrum aucheri* and *Artemisia aucheri*, and in the second stand (hereafter coded *Prangos uloptera2*), the co-dominant plant species were *Astragalus microcephalus*, *Acanthophyllum microcephalum* and *Poa bulbosa* (Table 1).

2.2. Soil sampling

Soil samples from each stand were collected from depths of 0 to 15 cm and 15 to 30 cm during spring 2011. Two key areas were selected within each stand to avoid pseudo-replication, yet the stands were close to each to other to limit co-varying factors other than co-dominant species (see for instance Oksanen, 2001). This area was considered as the representative of the entire habitat in that location (Heady and Child, 1994). In each key area, six transects were established: three parallel and three perpendicular to the slope. Three 4m×4m quadrats were established at the beginning, the middle and the end of each transect. In each quadrat, 10 soil cores were collected at random, to a depth of 30cm, with a 5cm diameter auger (each core was divided into two sub-cores (0-15 and 15-30cm) and sub-cores were then pooled per depth for each quadrat. All of the soil samples were immediately transferred to a cooled, insulated container for transport to the laboratory and were stored at 4ºC until they were processed. The samples were sieved, the roots and coarse gravel (>5mm) were removed by sieving, and the <5mm soil was used to examine the effects of vegetation composition on soil parameters.

2.3. Soil and data analysis

Organic C was determined by the Loss of Ignition method (Lal et al., 2001) and total soil N by wet oxidation using the Kjeldahl method (Zagal et al., 2009). The POM was determined by physical fractionation (Cambardella and Elliot, 1992). Twenty-five grams of air-dried soil samples were dispersed with 100 ml of 5 g/l of sodium hexametaphosphate. The soil solution mixture was shaken for 1 h at high speed (=500 rpm) on an end-to-end shaker and poured over a 0.053 mm sieve with several deionized water rinses. The soil remaining on the sieve was back washed into a pre-weighed aluminium dish and dried at 60ºC for 24 h, then ground and analyzed for C and N (see also Handayani et al., 2009).

Aggregate size distribution was determined using wet sieving with screen diameters of 0.25 and 0.50 mm. The range of micro-aggregates and macro-aggregates was between 0.053 to 0.25 mm and 0.25 to 0.50 mm, respectively. Soils samples were submersed in water on the largest screen for 5 min before sieving commenced. Soils were sieved under water by gently moving the sieve 3 cm vertically through water contained in a shallow pan, 50 times over period of 2 min. Material remaining on the sieve was transferred to an aluminium container and dried at 60ºC in a forced-air oven then weighed and measured for C (Elliot and Cambardella, 1991).

Soil parameters of the two stands were compared using t-tests at the 5% significance level.

3. Results

3.1. Total organic carbon and nitrogen content in soils

Total C in the upper layer (0-15 cm soil depth) was significantly higher in the *Prangos uloptera2* stand compared to *Prangos uloptera1* (9.5 g/kg vs. 3.6 g/kg, respectively; t=-25.34, $p<0.01$) (Fig. 1A). At the 15 to 30 cm soil depth, the difference between the two stands was significant with 6.4 g/kg and 3.2 g/kg in *Prangos uloptera2* and *Prangos uloptera1*, respectively (t=-16.62, $p<0.01$).

Total N content in the upper layer increased from 0.4 g/kg to1.2 g/kg and in lower layer from 0.4 g/kg to 1.0 g/kg in *Prangos uloptera1* and *Prangos uloptera2*, respectively (t= -21.65, p<0.01; t=-19.38, p<0.01) (Fig. 2A).

3.2. Labile SOM fractions

Soil POM-C and also POM-N were generally higher in the *Prangos uloptera2* (Fig. 1B and Fig. 2B) stand compared to the other treatment. POM-C contents in *Prangos uloptera1* and *Prangos uloptera2* were 0.3 g/kg and 2.9 g/kg in the upper soil layer, respectively (t=-88.33, p<0.01). POM-C contents in *Prangos uloptera1* and *Prangos uloptera2* were 0. 7 g/kg and 1.5 g/kg in the deeper soil layer, respectively (t=-23.82, p<0.01). POM-N contents in both depths of the *Prangos uloptera2* stand were significantly higher than the *Prangos uloptera1* stand (0.4 g/kg and 0.2g/kg vs. 0.1g/kg and 0.1g/kg) (t=-26.84, p<0.01; t=-2.86, p<0.01)(Fig. 2B).

3.3. Aggregate distribution and carbon associated with aggregate size classes

There was a significantly smaller proportion of soil in macro-aggregates in *Prangos uloptera2* compared to *Prangos uloptera1* in both depths (upper layer: t=21.68, p<0.01; lower layer: t= 35/29, p<0.01) (Fig. 3A). Conversely, there was a significantly smaller proportion of soil in micro-aggregates in *Prangos uloptera1* compared to *Prangos uloptera2* in both depths of the upper layer (t=-21.68, p<0.01; lower layer: t= -28/48, p<0.01)(Fig. 3B).

Carbon content was significantly greater for each aggregate in *Prangos uloptera2* than *Prangos uloptera1* in both depths (Fig. 4A and 4B). The greatest significant difference (t=-14.63, P<0.01) in C content between Prangos *uloptera1* and *Prangos uloptera2* was in the macro-aggregates at the 0-15cm soil depth (0.26 to 1.13g/kg) compared to the micro-aggregates (0.86 to 1.54g/kg) (t=-18.80, p<0.01) (Fig. 4A and 4B).

4. Discussion

Results of this study showed that the total nitrogen, organic carbon and C associated with micro- and macro-aggregates were significantly higher in the *Prangos uloptera2* than the *Prangos uloptera1* community. We suggest that the two co-dominant plant species, with the higher canopy cover in *Prangos uloptera2* compared to the other stand, are responsible for this difference. The first species is *Acanthophyllum microcephalum* with a mean percentage cover of 17% in *Prangos uloptera2* compared with 4% in *Prangos uloptera1*. This species is a relatively large shrubby plant with high production in the Iranian range lands (Arzani et al., 2005). Nutrient accumulation as fertile islands beneath shrubs in grassland is common and provides opportunities for carbon and nitrogen sequestration in arid and semi-arid regions (e.g. Jackson et al., 2002). Mcclaran et al. (2008) showed that the SOM and total nitrogen accumulation was 80-750% greater in the beneath *Prosopis velutina* than in the open grassland. The second species with a high proportion of canopy cover in *Prangos uloptera2* was a leguminous species, *Astragalus microcephalus* which is a deciduous shrub growing to 0.5 m. It produces woody stems which tend to die back almost to the base each winter. This species has a symbiotic relationship with certain soil bacteria, which form nodules on the roots and fix atmospheric nitrogen (Huxley and Griffiths, 1992). Increasing N to the soil via N_2 fixation and plant organic inputs by this species may increase total N and C in the soils of *Prangos uloptera2*. Moreover, the tiny dense shallow roots of *Poa bulbosa* (Barnhart, 1895) may increase organic carbon in the *Prangos uloptera2* community. Previous studies also showed that soil carbon additions are governed by the volume of fibrous roots per unit of soil and the rate of growth of gramineae. The greater the number of active green leaves and active plant roots, the more carbon is captured from the air, and thus translocated through the plant and exuded into the soil (Jones, 2006; Kadović, 2012).

Soil C and N contents play a crucial role in sustaining soil and environmental quality (Su, 2007). In addition, knowledge of the effects of vegetation type and plant composition on SOM status is essential in natural habitats when considering conservation strategies based on higher carbon sequestration in these habitats. In this study, SOM had spatial variation in natural habitats, driven by the plant composition, and mainly by the difference in co-dominant species. Our findings are similar to those obtained by a few other studies. Monokrousos et al. (2004) showed that soil samples collected from sites with dimorphic species share common soil properties, whereas sites with different evergreen species showed distinct soil properties for each species. Roukos et al. (2011) found that SOM is significantly affected by the plant community and Bu et al. (2012) observed that the C/N ratio in density fractions were higher in coniferous areas than in alpine meadows. Oueslati et al. (2013) found that organic carbon was significantly affected by the plant species in the ground vegetation. Contrary to our results, Mendham et al. (2004) and Peichl et al. (2012) reported that changing the plant community and species by the afforestation of grassland had no significant impact on total soil C and N stocks.

However, studies on the effect of vegetation on SOM are scarce in natural habitats. In managed lands and cultivated areas, practices may also have wide-ranging impacts on soil C and N (Marcos et al., 2006; Luan et al., 2010; Fallahzade and Hajabbasi, 2010; Beedy et al., 2010). SOM may also be affected by climate, soil texture, nutrient statuses and time since the land management was initiated (Franzluebbers and Arshad, 1997; De Koning et al, 2003).

Labile fractions of SOM have already been suggested as important indicators of impacts on soil resulting from management practices (e.g. Handayani, 2004; Sequeira et al., 2011). This study also revealed the sensitivity of labile fractions of soil to natural vegetation changes. The differences in vegetation between *Prangos uloptera1* and *Prangos uloptera2* increased POM-C and POM-N, 89.6% and 75.0% in topsoil (0-15cm depth), respectively. While, total C and N in

the upper layer were increased 62.1% and 66.6%, respectively by vegetation differences between *Prangos uloptera1* and *Prangos uloptera2*. The ranges of quantities of POM-C and POM-N in the study area were 0.3 to 2.9 g/kg and 0.1 to 0.4 g/kg, respectively. These values are in the range reported by Gupta et al., (1994) (POM-N: 0.13 to 0.29 g/kg) and Ordraogo et al., (2006) (POM-N: 0.11 to 0.27 g/kg) but lower than those observed by Handayani et al. (2009) (POM-C: 1.92 to 4.02 g/kg and POM-N: 0.28 to 0.96 g/kg). Generally, the composition of POM consists mainly of root fragments (Cambardella and Elliot, 1992). Thus, significantly different levels of POM-C and POM-N between the two stands in this research would suggest differences in root biomass. It can be concluded that the leguminous species (*Astragalus microcephalus)* in *Prangos uloptera2* probably promotes more decomposition and root regeneration due to additional N, which may increase root contributions to POM (see also Tisdall, 1991, Braakhekke et al. 2013).

Here, the significant difference in macro-aggregates and micro-aggregates between the two stands may be due to the effect of the dense micro-roots of the leguminosae (*Astragalus microcephalus*) and the gramineae (*Poa bulbosa*) in *Prangos uloptera2*. Poaceae generally have a system of extensive tiny roots (Barnhart, 1895) that enfolds the soil ingredients like a trap and increases permanent aggregates in water. *Prangos uloptera2* probably had fewer strong roots than *Prangos uloptera1*, thus decreasing the number of macro-aggregates. In accordance with our results, Liao et al. (2006) showed that, compared to herbaceous species (e.g. *Eragrostis* sp.), a woody species (*Prosopis glandulosa*) increased the relative proportions of the free light fraction and macro-aggregates and decreased the micro-aggregate size fraction. The reduction of macro-aggregates in soils under human activity has already been documented by previous works in which long-term cultivation has decreased the length and mass of fine roots and SOM, resulting in a reduction of macro-aggregates (Tisdall and Oades, 1980).

5. Conclusion

This study reveals that changes in the co-dominance of plant species can have significant adverse effects on the proportion of SOC in natural grasslands. In conservation projects based on higher carbon sequestration, the manager should consequently identify the plant species which can absorb and add carbon into soil at a higher rate and try to improve their spatial extent.

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Figure captions

Fig. 1. Physical fractionation of soil organic matter for C (A) under two *Prangos uloptera* stands. POM-C is particulate organic matter C (B). Different successive letter indicate significant differences (at α=0.05) between the two stands. Co-dominant species in *Prangos ulopteral1* were *Pterpyrum aucheri* and *Artemisia aucheri*, and in *Prangos uloptera2* were *Astragalus microcephalus*, *Acanthophyllum microcephalum* and *Poa bulbosa*.

Fig. 2. Physical fractionation of soil organic matter for N (A) under two *Prangos uloptera* stands. POM-N is particulate organic matter N (B). Different successive letter indicate significant differences (at α=0.05) between the two stands. Co-dominant species in *Prangos ulopteral1* were *Pterpyrum aucheri* and *Artemisia aucheri*, and in *Prangos uloptera2* were *Astragalus microcephalus*, *Acanthophyllum microcephalum* and *Poa bulbosa*.

Fig. 3. Aggregate size distribution (macro- and micro-aggregates: A and B, respectively) under two *Prangos uloptera* stands. Different successive letter indicate significant differences (at α=0.05) between the two stands. Co-dominant species in *Prangos ulopteral1* were *Pterpyrum aucheri* and *Artemisia aucheri*, and in *Prangos uloptera2* were *Astragalus microcephalus*, *Acanthophyllum microcephalum* and *Poa bulbosa*.

Fig. 4. Soil C fractions associated with aggregate size under two *Prangos uloptera* stands. Different successive letter indicate significant differences (at α=0.05) between the two stands. Co-dominant species in *Prangos ulopteral1* were *Pterpyrum aucheri* and *Artemisia aucheri*, and in *Prangos uloptera2* were *Astragalus microcephalus*, *Acanthophyllum microcephalum* and *Poa bulbosa*.

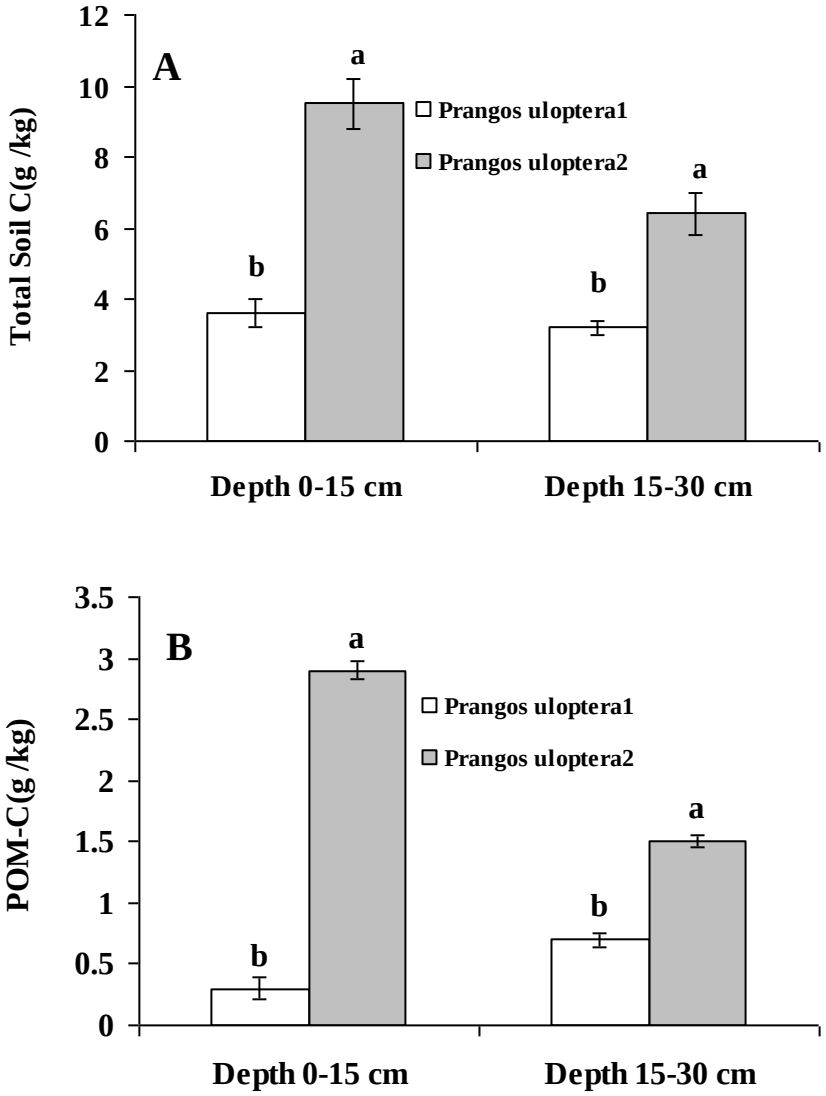
A
Total Soil C(g /kg)
12
10
8
6
4
2
0
a
b
b
a
Prangos uloptera1
Prangos uloptera2
Depth 0-15 cm
Depth 15-30 cm
B
POM-C(g /kg)
3.5
3
2.5
2
1.5
1
0.5
0
a
b
b
a
Prangos uloptera1
Prangos uloptera2
Depth 0-15 cm
Depth 15-30 cm

Figure 1

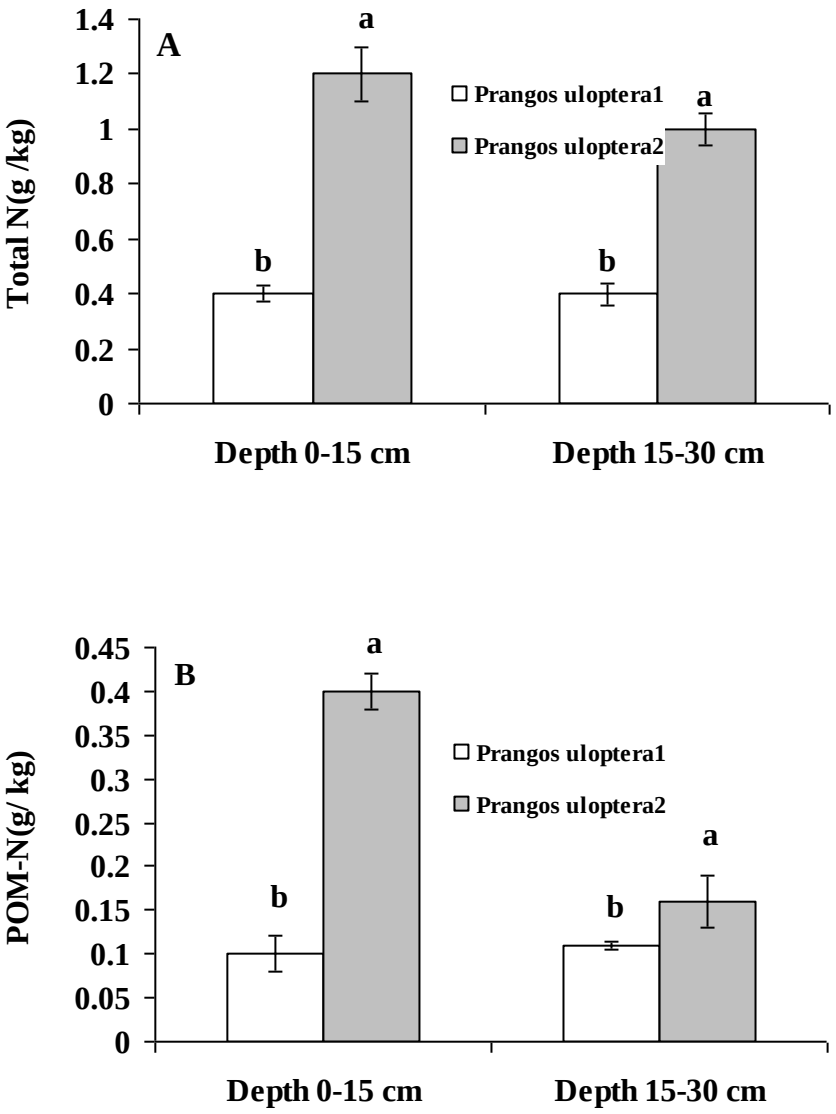
A
Total N(g /kg)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
a
b
Prangos uloptera1
Prangos uloptera2
Depth 0-15 cm
Depth 15-30 cm
B
POM-N(g/ kg)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
a
b
a
b
Prangos uloptera1
Prangos uloptera2
Depth 0-15 cm
Depth 15-30 cm

Figure 2

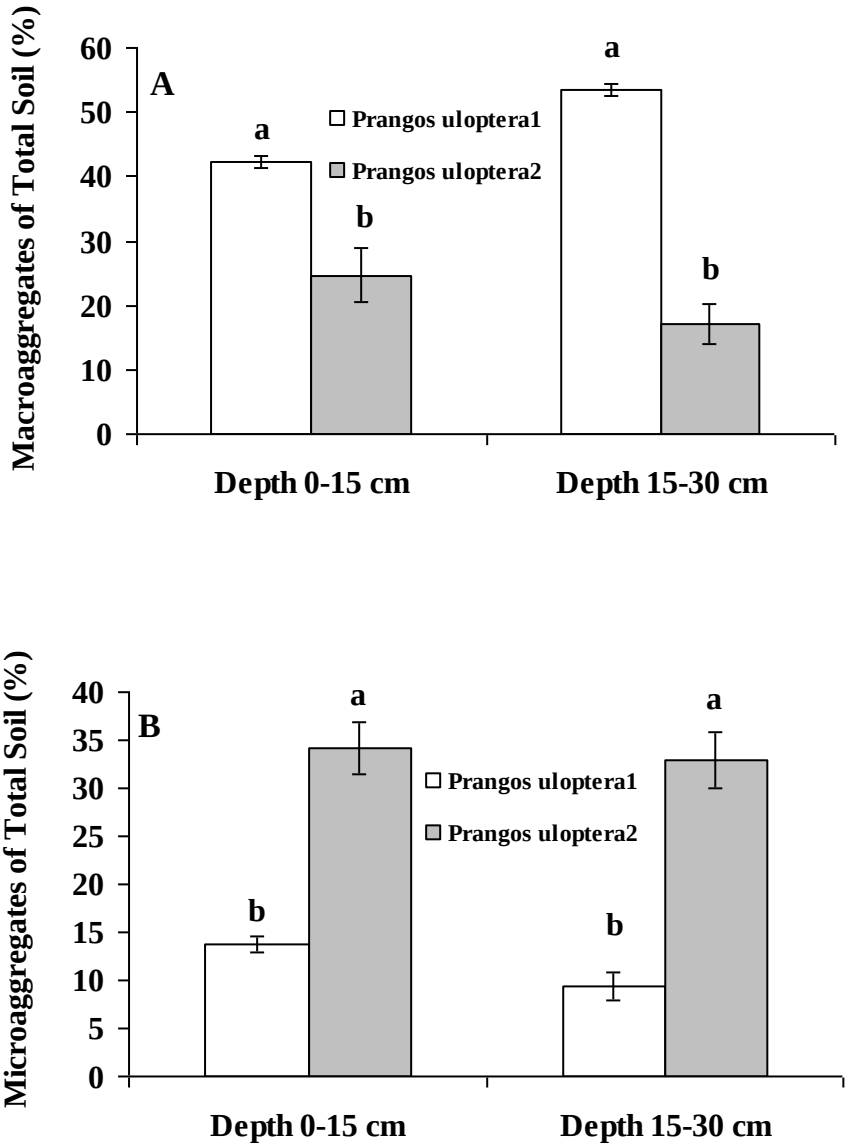
A
Macroaggregates of Total Soil (%)
60
50
40
30
20
10
0
a
b
a
b
Prangos uloptera1
Prangos uloptera2
Depth 0-15 cm
Depth 15-30 cm
B
Microaggregates of Total Soil (%)
40
35
30
25
20
15
10
5
0
b
a
b
a
Prangos uloptera1
Prangos uloptera2
Depth 0-15 cm
Depth 15-30 cm

Figure 3

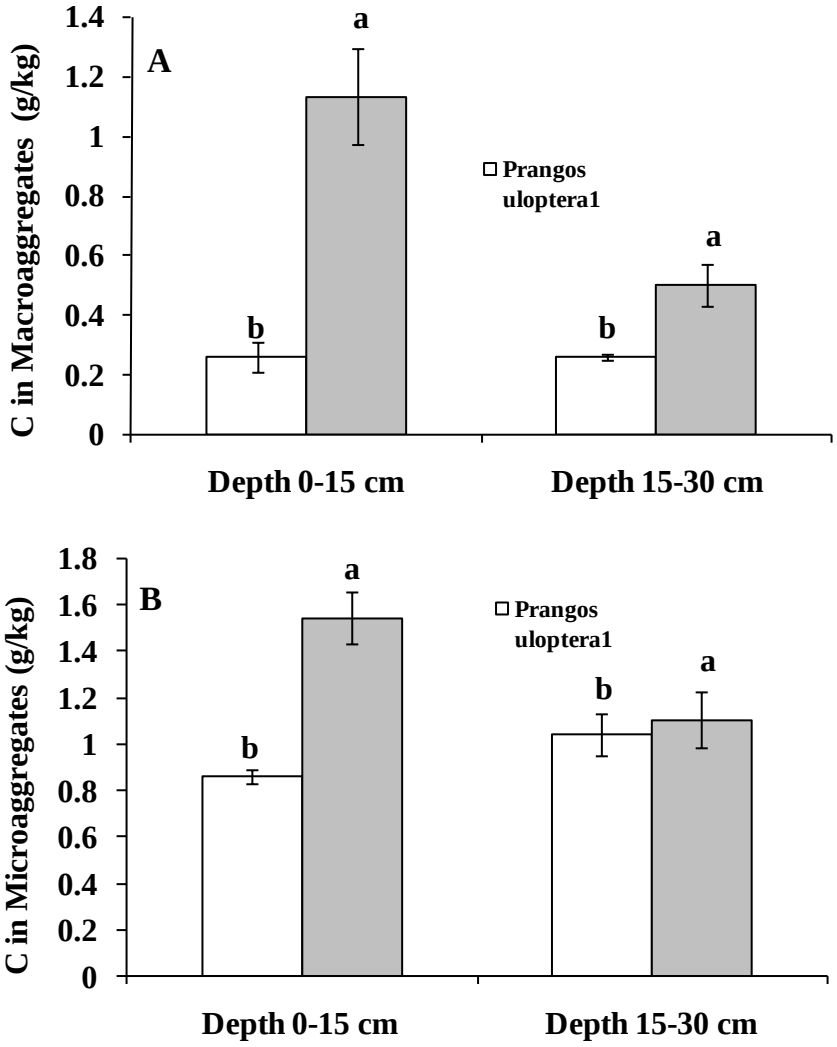
A
C in Macroaggregates (g/kg)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
a
Prangos uloptera1
Depth 0-15 cm
Depth 15-30 cm
B
C in Microaggregates (g/kg)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
b
a
Prangos uloptera1
Depth 0-15 cm
Depth 15-30 cm

Figure 4