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Distribution pattern of amidohydrolase activities among soil aggregates: Effect of soil aggregates isolation methods

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ABSTRACT

The effect of aggregate isolation methods on distribution patterns of N-cycling enzymes within soil aggregate is not well understood. In this study, the effects of wet and dry sieving methods on organic C (OC) content and amidohydrolase activities (Urease, L-glutaminase and L-asparaginase) were determined for six aggregate sizes (4-2, 2-1, 1-0.5, 0.5-0.25, 0.25-0.05 and < 0.05 mm) isolated from five grassland soils. The distribution of wet-sieved aggregates was skewed toward microaggregates (0.25–0.05 mm) and silt and clay fractions (< 0.05 mm), while the contrary was found with dry sieving. Wet-sieved macroaggregates (> 0.25 mm) had higher OC content and potential amidohydrolase activities than other aggregate sizes but not a specific size of macroaggregates was consistently higher in all soils studied. No significant differences in OC and amidohydrolase activity were also observed between dry-sieved macroaggregates. Although dry-sieved 0.25–0.05 mm fraction had generally higher amidohydrolase activities than the other aggregate sizes, the distribution pattern of urease (URE) activity within dry-sieved aggregates was different among soils studied. Unlike the dry sieving method, microaggregates and silt and clay fractions separated by wet sieving had a major contribution to the total OC content and amidohydrolase activities in all soils. Both sieving methods altered the amidohydrolase activities, causing either losses or even increases depending on the soil and the enzyme studied. The significant difference between grassland soils in terms of OC content and enzyme activity was observed in wet- and dry-sieved aggregates, although it was more pronounced in wet-sieved large macroaggregates. Overall, sieving methods resulted in different OC content and amidohydrolase activities in soil aggregates, however, wet sieving showed greater ability to reveal significant differences in terms of aggregate potential enzyme activity compared to dry sieving. Wet sieving was also most capable to examine long-term changes in organic matter and enzyme activity between soil types.

1. Introduction

There is still a need to improve our understanding of biological functioning in soils. For this, it requires taking into account the spatial heterogeneity of soil ecosystem both at a macro scale (i.e., bulk soil) and at a micro scale level (i.e., aggregate and/or particle size). Soil aggregates, and specifically intra-aggregate pore space, create a mosaic of microenvironments, differing in their physical, chemical and biological characteristics, representing as many different habitats for the biotic component (Ranjard et al., 2001).

There are many approaches for soil aggregate isolation differing in the form of mechanical energy input, soil pretreatment before sieving, sieve loading and oscillation rate for sieving that may influence the aggregate size distribution (Kemper and Koch, 1966). In general, the soil fractionation methods are divided into two methods: wet and dry sieving (Bach and Hofmockel, 2014; Blaud et al., 2017). The wet sieving is a method involves immersing dry-sieved aggregates in water and then sieving by hand or machine (Elliott, 1986). It disintegrates soil aggregates by increasing water pressure on the air trapped inside particle pores, osmotic swelling forces or water solubility of binding agents. The method has been widely used to investigate aggregate stratification of soil bacterial communities (Davinic et al., 2012), microbial abundance and diversity (Blaud et al., 2017), microbial biomass (Gupta and Germida, 1988; Jiang et al., 2011) and microbial activity (Bach and Hofmockel, 2014; Fansler et al., 2005). However, the possibility of microbial habitat disruption, potential alteration of the microbial community composition, activity and abundance and exclusion of water-soluble compounds in aggregates during wet sieving has led to consider alternative fractionation methods, such as dry sieving (Mendes et al., 1999; Miller et al., 2009; Sainju, 2006). Dry sieving has also been suggested to measure wind erosion in arid and semiarid regions (Sainju, 2006). Dry sieving involves shaking aggregates in a nest of sieves by

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Table 1
Physicochemical properties and enzyme activities of bulk soils studied.

Soil	Location	Slope position	Slope degree	OC	TN	pH	EC	$CaCO_3$	Clay	Silt	Sand	LGL	LAS	URE
			%	g kg^{-1}			ds m^{-1}	%	g kg^{-1}			mg NH_4^+–N kg^{-1} h^{-1}		
A	32° 45′665″N 50° 11′ 529″E	Summit	1	17.8 ± 0.24	1.4 ± 0.06	8.1 ± 0.03	0.40 ± 0.01	4.5 ± 0.0	132 ± 15.2	707 ± 21.4	161 ± 16.4	69.8 ± 7.8	7.83 ± 1.04	40.1 ± 4.6
B	32° 45′ 676″N 50° 11′ 518″E	Upper slope	25	14.9 ± 0.24	1.1 ± 0.04	7.9 ± 0.02	0.31 ± 0.00	2.0 ± 0.4	97 ± 3.3	706 ± 16.3	197 ± 13.1	34.9 ± 2.7	6.29 ± 0.34	33.4 ± 3.3
C	32° 45′ 745″N 50° 11′ 471″E	Middle slope	36	12.0 ± 0.37	0.9 ± 0.03	8.0 ± 0.07	0.28 ± 0.00	3.3 ± 0.5	107 ± 8.2	747 ± 10.6	146 ± 31.3	22.6 ± 5.5	2.51 ± 0.10	17.2 ± 2.8
D	32° 45′ 751″N 50° 11′ 456″E	Lower slope	15	10.6 ± 0.61	0.9 ± 0.02	8.2 ± 0.01	0.26 ± 0.00	5.8 ± 0.6	232 ± 4.4	667 ± 61.9	110 ± 2.7	14.0 ± 1.5	1.67 ± 0.42	16.5 ± 2.2
E	32° 45′ 750″N 50° 11′ 423″E	Toe slope	5	14.2 ± 0.67	1.2 ± 0.02	8.0 ± 0.05	0.41 ± 0.02	4.3 ± 0.2	86 ± 5.4	818 ± 7.5	96 ± 7.1	34.2 ± 4.1	5.03 ± 0.1	35.2 ± 7.2

Note: OC, organic carbon; TN, total nitrogen; EC, electrical conductivity; URE, urease activity; LGL, L-glutaminase activity; LAS, L-asparaginase activity. Values correspond to the mean samples (n = 3) and the standard deviation.

hand or machine. Therefore, the different microbial response to wet and dry sieving can be expected when the mechanical energy inputs differ greatly between the sieving methods. Despite such discrepancies, both methods are used by researchers making it difficult to compare results of chemical and especially microbial properties in soil aggregates. Therefore, studying and defining a standard method for aggregate separation of various soils should be prioritized.

There are many studies regarding biological properties in wet- or dry- sieved aggregates, however, comparative studies regarding variations of biological properties among soil aggregates as affected by fractionation methods are scarce and also limited to one or two soils studied (Ashman et al., 2003; Bach and Hofmockel, 2014; Blaud et al., 2017). Perez Mateos and Gonzalez Carcedo (1985) reported that enzyme activities among soil aggregates were affected by various wet sieving methods. When the undisturbed soil was fractionated, enzyme activities were dominant in the largest aggregates. The enzyme activities were predominantly located in small aggregate fractions when wet sieving was carried out after the soils were dispersed in water or disrupted mechanically. This activity results in losses of macroaggregates due to disruption by water. Bach and Hofmockel (2014) also reported different distribution patterns of N-acetyl-glucosaminidase, β-glucosidase, β-xylosidase and cellobiohydrolase activities within wet, dry and optimal moisture sieved aggregates obtained from two contrasting land uses. The potential enzyme activities were shown to be overestimated due to wet sieving. However, they concluded that wet sieving is most useful to examine long-term changes in soil organic matter and microbial activity between soil types. Blaud et al. (2017) determined the effect of wet and dry sieving methods on bacterial diversity, and abundance of microorganisms involved in N cycling among soil aggregates obtained from two land uses (cropland and grassland). Their results showed that wet sieving led to increase gene abundance and significant differences in bacterial community composition between fractions in grassland. However, Sainju (2006) observed that wet sieving can increase or decrease microbial biomass C depending on soil type and fraction in comparison to dry sieving.

The effect of sieving methods on enzyme activities remains still largely unknown. Further studies are needed to assess the fractionation methods across a large number of soils and land use and also for various enzymes. In this study, the effect of wet and dry sieving methods on amidohydrolase activities [urease (EC 3.5.1.5), L-glutaminase (EC 3.5.1.2) and L-asparaginase (EC 3.5.1.1)], was determined for six aggregate sizes from different grassland soils. The enzymes participate in N mineralization process by increasing the hydrolysis of organic N compounds (Tabatabai, 1994). To our knowledge, there is no comparative study regarding the effect of sieving methods on amidohydrolase activities in soil aggregates. We hypothesized that soil fractionation methods influence the distribution pattern of amidohydrolase activities within soil aggregates and the effect of sieving methods on the enzyme activities is soil specific. Therefore, the objective of this study was to investigate how amidohydrolase activities within aggregates of different grassland soils are influenced by fractionation methods.

2. Materials and methods

2.1. Site description and soil sampling

This study was conducted in Fereydan hilly region, western of Isfahan province, central Iran (50° 11′E, 32° 45′N). The mean annual precipitation and air temperature at the site are 600 mm and 5° C, respectively. The area is dominated by hills and valleys with most slopes ranging between 1 and 50%. The grasslands of the area have been degraded due to overgrazing by sheep for the last 30 years. The vegetation is largely dominated by *Cousinia bachtiarica*, *Eryngium billardieri*, *Astragalus verus* and *Astragalus* spp. We observed that soil erosion and plant coverage were different along the hillslopes of various region of the grassland area which are known to influence on soil properties.