Effect of long-term fertilization on soil aggregate-associated dissolved organic nitrogen on sloping cropland of purple soil

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ABSTRACT

To investigate the effect of fertilization practice on dissolved organic nitrogen (DON) in aggregates, the study was conducted and involved four fertilization treatments: no fertilizer; mineral fertilizer (MF); pig manure matched with mineral fertilizer (MFP) and crop straw matched with mineral fertilizer (MFR). The results showed that DON content and storage were the highest in > 5 mm aggregates and were the lowest in < 0.25 mm aggregates. Compared with MF, MFP and MFR significantly increased DON contents in > 5 mm by 404.7% and 184.4%. In comparison with MFR, DON content and storage in > 5 mm aggregates for MFP were significantly enhanced by 77.5% and 75.0%. A significantly positive linear correlation relationship between DON content and microbial biomass carbon content was observed in aggregates (R^2 = 0.84; P < 0.01). The results suggest that pig manure matched with mineral fertilizer is a preferred strategy for retaining DON nutrient due to enhanced microbial biomass in aggregates on sloping upland of purple soil.

Keywords: cropland; mineral fertilizer; manure; crop straw; microbial biomass

Dissolved organic nitrogen (DON) is considered to play an important role in biogeochemical cycling processes in terrestrial ecosystem (Cookson and Murphy 2004, Jones et al.2004). It is an essential source of available N for microorganisms and plants (Neff et al. 2003).

Aggregates, which are the major important component in soil, play an important role in maintaining soil fertility and nitrogen nutrients (Bronick and Lal 2005, Peregrina et al. 2010, Guo and Wang 2013). Several researchers reported that there was a close relationship between nitrogen nutrients and soil aggregates in cropland. For instance, Sainju et al. (2003) showed that total organic nitrogen (TON), microbial biomass nitrogen (MBN) and particular organic nitrogen (PON) contents were greater in the < 0.25 mm soil aggregation. Liu et al. (2007) reported that TON content increased in the order of > 5, 2–5, 1–2 and 0.25–1 mm and decreased from 0.25–1 mm to < 0.25 mm aggregates, while there was no obvious distribution pattern for the content of NH_4^+-N and NO_3^--N. However, distribution of DON in aggregates has not been clearly understood. Hence, it is necessary to differentiate and quantify the DON in soil aggregates, which is fundamental to understand nitrogen nutrients in

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agricultural ecosystem. Fertilizer application was an essential management to enhance crop yield in agricultural ecosystem. Meanwhile, crop straw or amendment of organic matter through fertilization are considered as major DON sources in arable soils (Qualls et al. 2002), which also greatly influence soil aggregates distribution.

Purple soils are widely distributed throughout the hilly parts of southwestern China and have a total area of 260 000 km^2 (Li et al. 1991). Sloping cropland distributed widely in the area has been degraded by nitrogen nutrients loss due to serious water erosion. Therefore, two main objectives of this study were (1) to examine the distribution of DON in soil aggregates and (2) to determine the effects of different fertilization practices on DON content and storage.

MATERIAL AND METHODS

Site and soil. The experiment site is located in the Yanting Agro-Ecological Station of Purple Soil (31°16'N, 105°28'E) at an altitude of 400–600 m in the central Sichuan Basin, Southwest China. The experimental site has a moderate subtropical monsoon climate with an annual mean temperature of 17.3°C and mean precipitation of 826 mm over the past twenty years (Zhu et al. 2009). There are 5.9, 65.5, 19.7 and 8.9% rainfall occurred in spring, summer, autumn and winter, respectively, during the total annual precipitation. Annual precipitation in this study was 892 mm in 2010, 1061 mm in 2011 and 1080 mm in 2012, respectively (Figure 1). The soil is called purple soil and classified as a Pup-Orthic Entisol in the Chinese Soil Taxonomy and an Entisol in the U.S. Soil Taxonomy due to its color (Gong 1999).

Experimental design and treatments. A long-term field experiment was initiated in 2003. The experiment includes two crops per year, wheat (from October to May next year) and maize (from May to September). The treatments were: no fertilizer (CK); mineral fertilizer (MF); mineral fertilizer combined with pig manure (MFP); mineral fertilizer combined with crop straw residue (MFR), respectively. The treatments were laid out in a randomized block design with three replications. The net plot size was constructed with an area of 8 m (length) by 4 m (width), a 7° slope gradient, and 60 cm soil depth. 130 and 150 kg N/ha were applied in wheat and maize growing seasons, respectively. Forty percent of total N applied was conversed from pig manure or crop straw residue (Table 1). 39 kg mineral phosphorous (P) and 30 kg potassium (K) per hectare per crop were applied as base fertilizers. The contents of organic carbon for pig manure, wheat straw and maize straw were 480.8, 451.6 and 463.1 g/kg. Total N contents were 32.7, 5.4 and 3.7 g/kg, respectively. The corresponding C/N ratios were 15, 84 and 125, respectively.

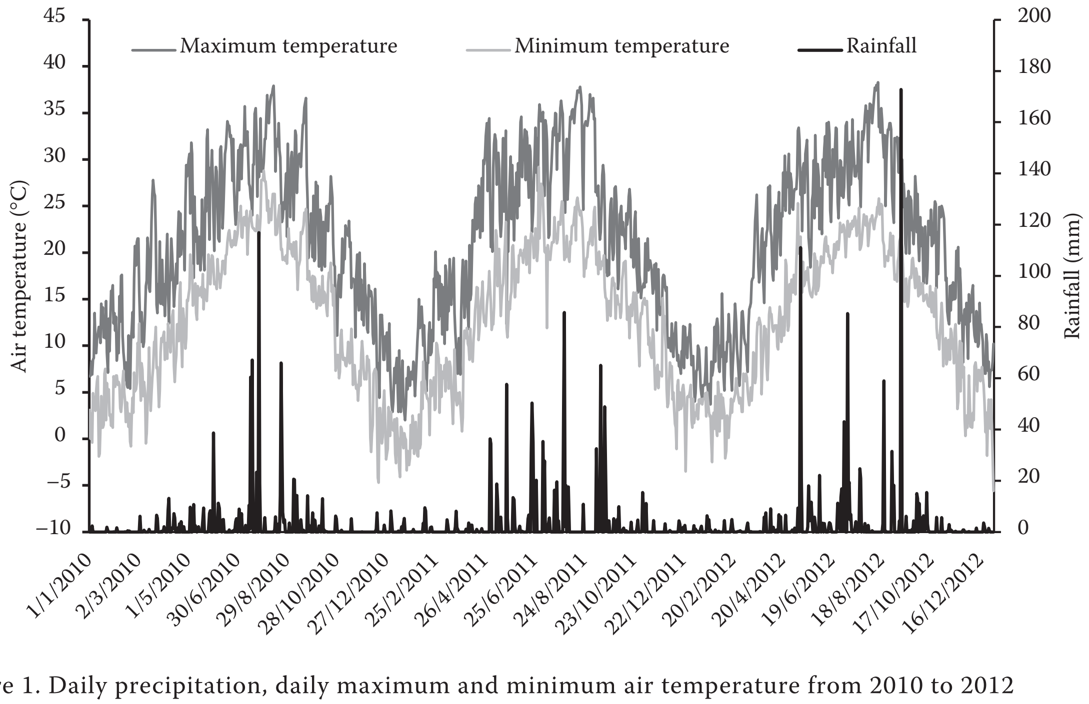

Figure 1. Daily precipitation, daily maximum and minimum air temperature from 2010 to 2012

Table 1. Fertilizer application rates in each treatment (kg/ha)

	Treatment	N application rate			Mineral P application rate	Mineral K application rate
		mineral N	straw	manure		
Wheat	CK	0	0	0	0	0
	MF	130	0	0	39	30
	MFP	78	0	52	39	30
	MFR	78	52	0	39	30
Maize	CK	0	0	0	0	0
	MF	150	0	0	39	30
	MFP	90	0	60	39	30
	MFR	90	60	0	39	30

CK – no fertilization; MF – mineral fertilizer; MFP – pig manure matched with mineral fertilizer; MFR – crop straw residue matched with mineral fertilizer

Soil aggregate separation. The four size aggregates were separated by dry-sieving the soil through a series of three sieves. There were > 5, 2–5, 0.25–2 and < 0.25 mm aggregates, respectively. The detailing processing of aggregates separated was adapted according to the description by Sainju et al. (2003). Sieves were shaken horizontally by hand for 3 min at 100 oscillations per min. Soils retained in sieves with size classes of > 5, 2–5, 0.25–2 and < 0.25 mm diameter were weighed and stored at 4°C until chemical analysis were measured.

Chemical analysis. Organic C, total N were determined by the methods described by Lu (1999). Microbal biomass carbon (MBC) content was determined by the fumigation-incubation method (Jenkinson and Powlson 1976). Inorganic nitrogen (NH_4^+-N plus NO_3^--N) in the extracts by 0.5 mol/L K_2SO_4 was measured by flow injection analyzer (Bran + Lubbe, Norderstedt, Germany). DON content in extracts were measured subtracting inorganic N (NH_4^+-N plus NO_3^--N) from dissolved total N (DTN) and DTN were determined by flow injection analyzer. Meanwhile, DON is calculated by the following equation:

$$DON_{storage\text{-}fraction} = DON_{con\text{-}fraction} \times M_{fraction}$$

Where: $DON_{storage\text{-}fraction}$ – storage in aggregates (mg/kg soil); $DON_{con\text{-}fraction}$ – DON content in aggregates (mg/kg fraction), and $M_{fraction}$ – mass of aggregates in whole soil (%).

Statistical analysis. All the statistical analysis was performed with SPSS 13.0 software package (SPSS, Inc., Chicago, USA). Significant differences were analyzed using the *LSD* test at significance level $P = 0.05$ or 0.01. Regression model and graph preparation were employed by Sigma plot 10.0 software (Systat Software, Inc., Chicago, USA).

RESULTS AND DISCUSSION

Aggregate mass distribution. The results showed that aggregate-size distribution was dominated by > 5 mm aggregates, which accounted for 40.6–61.8% of the whole soil (Figure 2). It was mainly due to more abundant hypha existed in > 5 mm aggregates (Guggenberger et al. 1999, Bedini et al. 2009). Because hypha related soil proteins is important binding agent for soil aggregates (Peng et al. 2011). Compared with MF, MFP and MFR significantly increased > 5 mm aggregates proportion, whereas obviously reducing the percentage of < 0.25 mm aggregates. Compared to MFP, > 5 mm aggregates proportion was signifi-

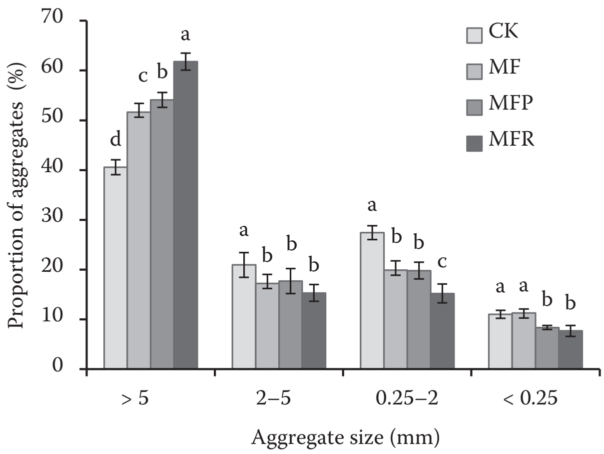

Figure 2. Proportion of aggregates. Vertical bars represent the standard deviation of the mean ($n = 3$). Columns with different small letters indicate significant differences at $P < 0.05$ or 0.01. CK – no fertilization; MF – mineral fertilizer; MFP – pig manure matched with mineral fertilizer; MFR – crop straw residue matched with mineral fertilizer

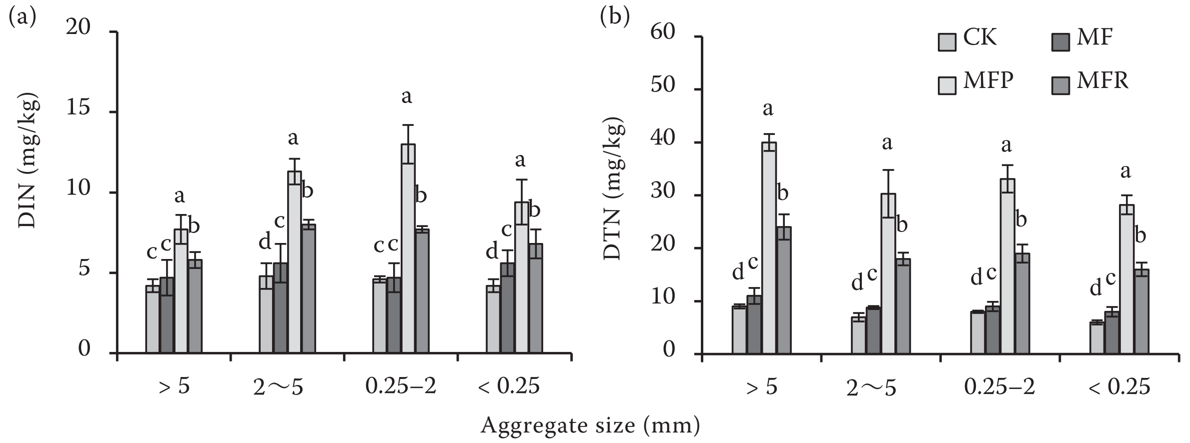

Figure 3. (a) DIN (NH_4^+ plus NO_3^-) and (b) total dissolved organic nitrogen (DTN) contents in aggregates. Vertical bars represent the standard deviation of the mean (n = 3). Columns with different small letters indicate significant differences at P < 0.05 or 0.01. CK – no fertilization; MF – mineral fertilizer; MFP – pig manure matched with mineral fertilizer; MFR – crop straw residue matched with mineral fertilizer

cantly increased by 14.2% under MFR, whereas no significant difference was found in the proportion of < 0.25 mm aggregates.

DTN and DIN contents in aggregates. DTN (total dissolved organic nitrogen) and DIN (NH_4^+ plus NO_3^-) contents in aggregates for all the fertilization treatments were shown in Figure 3. DTN content was highest in > 5 mm aggregates and was lowest in < 0.25 mm aggregates. The lowest DTN contents in aggregates were observed in CK, and the highest in MFP, following the order: MFP > MFR > MF > CK. DTN content in > 5 mm aggregates for CK was 9.0 mg/kg. By contrast, MF, MFP and MFR significantly enhance the DTN content by 22.2, 344.4 and 166.7%, respectively. Similarly, DIN contents in aggregates were greatly enhanced by MF, MFP and MFR, especially for MFP. The results suggested that both DTN and DIN contents in aggregates could be significantly improved by application of farmyard manure combined with mineral fertilizer.

Aggregate-associated DON contents and storages. DON content was calculated by subtracting DIN from DTN. For all the fertilization treatments, DON content was highest in > 5 mm aggregates and was lowest in < 0.25 mm aggregates (Table 2). In contrast to MF, DON contents in aggregates were significantly increased by MFP and MFR. DON contents in > 5 mm aggregates for MFP and MFR were 32.3 ± 1.6 and 18.2 ± 2.4 mg/kg and were significantly higher than MF. The effects of fertilizers on DON content were significantly different with the variation of fertilizer types. The quantity and quality of exogenous organic material affected soil microbial activity, thereby influencing soil organic nitrogen turnover (Marschner and Kalbitz 2003). It was due to less exogenous organic matter for MF treatment added into soil, thus resulting

Table 2. Dissolved organic nitrogen contents (mg/kg) and storages (mg/kg soil) in aggregates

Treatment	> 5 mm		2–5 mm		0.25–2 mm		< 0.25 mm	
	content	storage	content	storage	content	storage	content	storage
CK	4.9 ± 0.7^d	2.0 ± 0.4^d	2.2 ± 0.6^d	0.5 ± 0.2^c	3.4 ± 0.6^d	0.9 ± 0.2^c	1.8 ± 0.1^d	0.2 ± 0.03^d
MF	6.4 ± 1.5^c	3.3 ± 0.9^c	3.2 ± 0.3^c	0.6 ± 0.1^c	4.3 ± 0.9^c	0.9 ± 0.3^c	2.4 ± 0.9^c	0.3 ± 0.1^c
MFP	32.3 ± 1.6^a	17.5 ± 1.3^a	19.1 ± 4.5^a	3.4 ± 1.3^a	20.1 ± 2.6^a	4.0 ± 0.9^a	18.8 ± 1.8^a	1.6 ± 0.2^a
MFR	18.2 ± 2.4^b	10.0 ± 1.2^b	10.1 ± 1.1^b	1.5 ± 0.4^b	11.3 ± 1.7^b	1.7 ± 0.5^b	9.2 ± 1.3^b	0.7 ± 0.2^b

Mean ± SD; SD – standard deviation of the mean (n = 3). Different small letters indicate significant differences at P < 0.05 or 0.01. CK – no fertilization; MF – mineral fertilizer; MFP – pig manure matched with mineral fertilizer; MFR – crop straw residue matched with mineral fertilizer

Table 3. Microbal biomass carbon contents (mg/kg) in aggregates

Treatment	> 5 mm	2–5 mm	0.25–2 mm	< 0.25 mm
CK	284.5 ± 8.6^{d}	177.2 ± 6.5^{d}	182.8 ± 9.1^{d}	154.2 ± 7.8^{d}
MF	409.7 ± 20.1^{c}	323.9 ± 11.4^{c}	375.3 ± 11.2^{c}	303.1 ± 17.9^{c}
MFP	845.5 ± 30.2^{a}	610.5 ± 22.7^{a}	661.9 ± 27.6^{a}	542.3 ± 30.4^{a}
MFR	433.2 ± 18.6^{b}	368.4 ± 10.5^{b}	398.5 ± 15.3^{b}	321.8 ± 21.2^{b}

Mean ± SD; SD – standard deviation of the mean (n = 3). Different small letters indicate significant differences at P < 0.05 or 0.01. CK – no fertilization; MF – mineral fertilizer; MFP – pig manure matched with mineral fertilizer; MFR – crop straw residue matched with mineral fertilizer

in lower microbial activity for decomposing soil organic nitrogen (Qiao et al. 2010). In contrast, more exogenous organic matter stimulated microbial growth and reproduction under long-term application of pig manure or crop straw residue. Furthermore, DON contents in aggregates in MFP were significantly higher than that in MFR. It was mainly attributed to the difference of pig manure and crop straw residue C/N ratios. Several studies showed that soil microbial biomass was enhanced by lower C/N ratio organic amendments, thereby accelerating soil organic nitrogen transforming to DON (Saetre and Stark 2005, Liu et al. 2008). In the study, pig manure C/N ratio was 15:1 and crop straw residue C/N ratio was 105:1. The crop straw was more difficult to be utilized by microbe transforming soil organic nitrogen to DON compared with pig manure. Similar to DON content in aggregates, DON storage in > 5 mm aggregates varied from 2.0 ± 0.4 to 17.5 ± 1.3 mg/kg and was highest (Table 2). MFP and MFR significantly increased the storage of DON in > 5 mm aggregates by 432.9% and 243.6% compared with MF.

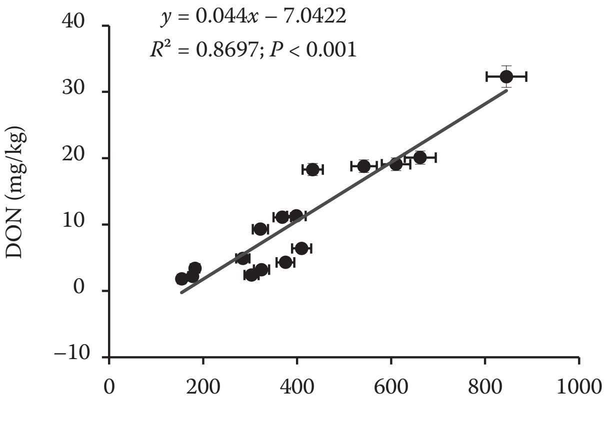

Figure 4. Relationship between microbal biomass carbon (MBC) and dissolved or-ganic nitrogen (DON) contents in aggregates. Vertical bars indicate the standard deviation of three different replicates

The results showed that both DON contents and storages in aggregates could be significantly improved by organic manure combined with mineral fertilizers, especially for farmyard manure.

Aggregate-associated MBC. MBC contents were highest in > 5 mm aggregates and lowest in < 0.25 mm aggregates for all the fertilization treatments (Table 3). MBC content in > 5 mm aggregates under CK treatment was 284.5 ± 8.6 mg/kg. Clearly, fertilization significantly increased MBC contents in aggregates. Furthermore, a positive relationship between MBC and DON contents in aggregates was observed (Figure 4, R^2 = 0.84; P < 0.001). The biomass of microbe is considered to be a major factor controlling DON content in soil, because DON can be directly produced by microbe turnover (Kalbitz et al. 2000). Burton et al. (2007) reported that DON can be derived from decomposition microbial debris in soil. Accordingly, higher DON contents were measured in MFP and MFR compared to MF, especially for MFP.

In conclusion, our findings suggest that > 5 mm aggregate is the dominant storage for DON on sloping upland. Long-term application of pig manure matched with mineral fertilization can improve DON content and storage, and the fertilization practice is a preferred strategy for retaining DON due to enhance microbial biomass in aggregates of purple soil in the Sichuan Basin, China.

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