Sun L, Jing H, Wang G, Liu G. 2018. Nitrogen addition increases the contents of glomalin-related soil protein and soil organic carbon but retains aggregate stability in a *Pinus tabulaeformis* forest. PeerJ 6:e5039 https://doi.org/10.7717/peerj.5039

Nitrogen addition increases the contents of glomalin-related soil protein and soil organic carbon but retains aggregate stability in a *Pinus tabulaeformis* forest

Lipeng Sun [1] , **Guoliang Wang** [1] , **Hang Jing** [1] , **Guobin Liu** Corresp. [1]

[1] Institute of soil and water conservation, Northwest A&F University, Yangling, Shaanxi, China

Corresponding Author: Guobin Liu
Email address: gbliu@ms.iswc.ac.cn

Background: Glomalin-related soil protein (GRSP) and soil organic carbon (SOC) contribute to the formation and stability of soil aggregates, but the mechanism by which global atmospheric nitrogen (N) deposition changes soil aggregate stability when it alters the distribution of GRSP and SOC in different aggregate fractions remains unknown. **Methods:** We used a gradient N addition (0–9 g N^{-2} y^{-1}) in *Pinus tabulaeformis* forest for 2 years in northeast China and then examined the changes in SOC contents, total GRSP (T-GRSP), and easily extractable GRSP (EE-GRSP) contents in three soil aggregate fractions (macro-aggregate: >250 μm, micro-aggregate: 250–53 μm, and clay–silt aggregate: <53 μm) and their relationship with aggregate stability. **Results:** (1) The soil was dominated by macro-aggregates. Short term N addition had no significant effect on mean weight diameter (MWD) and geometric mean diameter (GMD). (2) GRSP varied among aggregate fractions, and N addition had variable effects on the distribution of GRSP in aggregate fractions. The EE-GRSP content in the macro-aggregates increased initially and then decreased with increasing N addition levels, having a peak value of 0.480 mg/g at 6 g N^{-2} y^{-1}. The micro-aggregates had the lowest EE-GRSP content (0.148 mg/g) at 6 g N^{-2} y^{-1}. Furthermore, the T-GRSP content significantly increased in the aggregate fractions with the N addition levels. (3) The macro-aggregate had the highest SOC content, followed by the micro-aggregate and the clay–silt aggregate had the lowest SOC content. N addition significantly increased the SOC content in all the aggregate fractions. (4) GRSP and SOC contents were not significantly correlated with MWD. **Conclusion:** The distributions of GRSP and SOC varied with aggregate fractions. GRSP and SOC contents increased by N addition, but this increase did not enhance aggregate stability in short term, and the improvement of stability might depend on binding agents and incubation time.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26649v1 | CC BY 4.0 Open Access | rec: 9 Mar 2018, publ: 9 Mar 2018

Author Cover Page

Nitrogen addition increases the contents of glomalin-related soil protein and soil organic carbon but retains aggregate stability in a *Pinus tabulaeformis* forest

Lipeng Sun[1], Guoliang Wang[1], Hang Jing[1], Guobin Liu[1]*

[1] Institute of Soil and Water Conservation, Northwest A&F University, Yangling,712100, Shaanxi Province, China

First author:
Lipeng Sun
e-mail: lipengsun@nwafu.edu.cn

Guoliang Wang
e-mail:glwang@nwsuaf.edu.cn

Hang Jing
e-mail: h8170166069@163.com

*** Correspondence:**
Guobin Liu
e-mail: gbliu@ms.iswc.ac.cn

Nitrogen addition increases the contents of glomalin-related soil protein and soil organic carbon but retains aggregate stability in a *Pinus tabulaeformis* forest

Abstract

Background: Glomalin-related soil protein (GRSP) and soil organic carbon (SOC) contribute to the formation and stability of soil aggregates, but the mechanism by which global atmospheric nitrogen (N) deposition changes soil aggregate stability when it alters the distribution of GRSP and SOC in different aggregate fractions remains unknown. **Methods:** We used a gradient N addition (0–9 g N^{-2} y^{-1}) in *Pinus tabulaeformis* forest for 2 years in northeast China and then examined the changes in SOC contents, total GRSP (T-GRSP), and easily extractable GRSP (EE-GRSP) contents in three soil aggregate fractions (macro-aggregate: >250 μm, micro-aggregate: 250–53 μm, and clay–silt aggregate: <53 μm) and their relationship with aggregate stability. **Results:** (1) The soil was dominated by macro-aggregates. Short term N addition had no significant effect on mean weight diameter (MWD) and geometric mean diameter (GMD). (2) GRSP varied among aggregate fractions, and N addition had variable effects on the distribution of GRSP in aggregate fractions. The EE-GRSP content in the macro-aggregates increased initially and then decreased with increasing N addition levels, having a peak value of 0.480 mg/g at 6 g N^{-2} y^{-1}. The micro-aggregates had the lowest EE-GRSP content (0.148 mg/g) at 6 g N^{-2} y^{-1}. Furthermore, the T-GRSP content significantly increased in the aggregate fractions with the N addition levels. (3) The macro-aggregate had the highest SOC content, followed by the micro-aggregate and the clay–silt aggregate had the lowest SOC content. N addition significantly increased the SOC content in all the aggregate fractions. (4) GRSP and SOC contents were not significantly correlated with MWD. **Conclusion:** The distributions of GRSP and SOC varied with aggregate fractions. GRSP and SOC contents increased by N addition, but this increase did not enhance aggregate stability in short term, and the improvement of stability might depend on binding agents and incubation time.

Keywords: Aggregate; Glomalin-related soil protein; Soil organic carbon; Nitrogen deposition;

Mean weight diameter

Introduction

Human activities have led to a rapid increase in global nitrogen (N) deposition, which is expected to increase by 70% in 2050 than in 2000 (Galloway et al. 2004). the increasing N deposition has some negative effects on soil quality. for example, N deposition can affect soil aggregate stability by changing plant growth rates(Bai et al. 2010), soil microbial activities (Keeler, Hobbie and Kellogg 2009), and soil chemical properties in forest communities (Yin et al. 2016). However, the effects of available N in soil on aggregate stability remains controversial (Liu et al. 2015, Tripathi, Kushwaha and Singh 2008, Zhong et al. 2017), especially with respect to the mechanisms by which available N in soil influences aggregate stability when it changes glomalin-related soil protein (GRSP) and soil organic carbon (SOC) in aggregate fractions.

GRSP can bind soil particles to form aggregates, and the stability of these aggregate may increase with increasing GRSP content (Wright and Upadhyaya 1998). Several studies showed that soil available N has variable effects on GRSP content in bulk soil. For instance, some researchers found that N addition significantly increased GRSP content in bulk soil (Zhang et al. 2015, Garcia et al. 2008), but Wuest found that N addition had no significant effects on GRSP content in bulk soil (Wuest et al. 2005). These inconsistent results may be related to the soil initial N content (Kathleenk, Katiem and Michellec 2007), as well as N addition level and duration (Garcia et al. 2008, Treseder 2004). For example, N addition could increase the growth of arbuscular mycorrhizal fungi (AMF) in the soil with low initial N content (Treseder and Allen 2002), thus it increases GRSP content in bulk soil because glomalin is mainly produced by AMF (Kathleenk et al. 2007). Meanwhile, N addition may inhibit the growth of AMF in soil with high initial N content and may thus decrease GRSP content in bulk soil (Treseder 2004). However, a recent study showed that N addition reduced plant fine root length and AMF infection rate in soil with low N content (Wang et al. 2017), indicating that N addition can decrease AMF and GRSP content even in such soil (Kathleenk et al. 2007). Therefore, exploring the relationship between GRSP and available N content in soil with low N content is necessary.

Soil is mainly composed of aggregates of various sizes. As far as we know, the mechanism underlying the influence of N addition on GRSP content in aggregates of variable size has not been reported yet. In some studies on farmland ecosystems, manure amendment was found to promote soil nutrient accumulation and microbial activities, considerably increasing GRSP contents in all soil aggregate fractions except micro-aggregates (Xie et al. 2015). In these farmland ecosystems, tillage management methods disrupts soil structure and reduces microbial biomass and extent of enzyme activity, resulting in the significant decrease of GRSP content in all soil aggregate fractions except clay–silt aggregates and small fractions of macro-aggregates (Wright, Green and Cavigelli 2007). Similar to manure amendment and tillage management, N addition may also alter soil nutrient accumulation and microbial activity (Treseder and Allen 2002, Treseder 2004). Thus, N addition may change the GRSP content in soil aggregates. We speculated that the effects of N addition on GRSP content in aggregate fractions at forest system might be similar to that of farmland ecosystems with manure amendment and tillage management, as both systems alter soil nutrient accumulation and microbial activity.

GRSP can bind soil particles to form aggregates. Thus, changing of GRSP in aggregate fractions can influence aggregate stability directly (Wright and Upadhyaya 1998). A large number of studies have shown that the correlation between GRSP and aggregate stability is significant and positive (Wright et al. 2007, Zhang et al. 2014). However, a recent study on the relationships between GRSP and aggregate stability in citrus rhizosphere found that EE-GRSP in 0.25–0.5 mm aggregates was significantly positively correlated with mean weight diameter (MWD), whereas the T-GRSP in 2.0–4.0 mm aggregates and EE-GRSP in 0.5–1.0 mm aggregates were significantly negatively correlated with MWD. However, the GRSP in other size aggregates (1.0–2.0 mm) was insignificantly correlated with MWD (Wu et al. 2013). These results indicated that the relationships between GRSP and aggregate stability may vary with the aggregate sizes. Thus, we hypothesize that low-level N addition may increase the GRSP content in all soil aggregate fractions except micro-aggregates, and high-level N addition may decrease the GRSP content in all soil aggregate fractions except clay–silt aggregates. The relationships between GRSP and aggregate stability will be different according to the aggregate sizes.

In most previous studies, N addition significantly increased SOC content in bulk soil (Nave et al. 2009), although some studies indicated that N addition considerably decreased or did not affect SOC content (Zak and Sinsabaugh 2004, Zhong et al. 2017). N addition has variable effects on SOC content in aggregate fractions and bulk soil (KH et al. 2014, Fonte et al. 2009). However, most studies were focused on the effects of N addition on SOC content in bulk soil, and changes in SOC content in aggregate fractions are rarely investigated (Pan et al. 2007). In agriculture ecosystems, N fertilization has a minimal effect on SOC content in aggregate fractions under a conventional maize cropping system (KH et al. 2014). In maize cropping systems utilizing organic residues, N addition significantly increases SOC content in clay–silt aggregates and has no significant effect on the SOC content in other size aggregates (Fonte et al. 2009). These inconsistent results may be explained by the following reasons: first, the physical protection on SOC were different in aggregate fractions, thus resulting in different accumulation and decomposition rates of SOC in aggregate fractions (BlancoCanqui and Lal 2004). Second, tillage management affects soil carbon (C) input. In a conventional maize cropping system, N addition has no significant effect on SOC content in aggregate fractions, but it has considerable effect on SOC content in clay–silt aggregates in a maize cropping system utilizing organic residues. The increased SOC content in clay–silt aggregates may be due to the input of organic residues (Fonte et al. 2009). At present, studies on the effect of soil available N on SOC in aggregates are mainly focused on farmland ecosystems, and no study on forestland system has been reported yet (Chen et al. 2017). We speculated that the effects of N addition on SOC content in aggregate fractions at forest system might be similar to that of maize cropping systems utilizing organic residues, as both systems return a large amount of plant biomass to soil. However, this speculation requires experimental validation. Recent studies found that the relationship between SOC and aggregate stability is uncertain. For example, SOC content has a significantly negative relationship with aggregate stability in red soil found in subtropical China (Li et al. 2005). Moreover, the relationships between SOC and aggregate stability were insignificant in different land use tropical Ultisol (Leelamanie and Mapa 2015). SOC content in aggregate fractions had different relationships with aggregate stability in rice–wheat rotation farmlands (Das et al. 2014). For example, SOC content in macro-aggregates is significantly positively correlated with aggregate stability, but SOC content in micro- and clay–silt aggregates significantly negatively correlated with aggregate stability. However, research on the relationships between SOC content in aggregate fractions and aggregate stability under N addition condition remains lacking. In the present study, we proposed that N addition significantly increases SOC content in clay–silt aggregates and minimal affect SOC content in other size aggregate fractions. The relationships between SOC content and aggregate stability are varied among aggregate fractions.

Two hypotheses were tested by determining GRSP and SOC contents in aggregate fractions and aggregate stability in a *Pinus tabulaeformis* forestland. This study is expected to determine the underlying mechanism of soil aggregate stability in response to N addition.

Materials and methods

Study area

The experiment was conducted in the Songyugou watershed in the Loess Plateau region, Shaanxi Province, China (35 390N, 110 060E). The region has a classic mainland monsoon-type climate with a mean annual precipitation of 584.4 mm, mean annual temperature of 9.7 °C, and frost-free period of 180 days. The soil is gray forest soil (Gray Luvisols, FAO soil classification) with a surface soil bulk density of 1.1 g/cm^3. The soil total phosphorus (P) content is 1.42 ± 0.38 g/kg^{-1}, and the soil pH is 8.6. *P. tabulaeformis* was planted in 1960. The current stand density is 1400 – 1800/hm^2. The average canopy density is 0.7. The average diameter at breast height (DBH) is 10.0 cm. The average height of the tree is 11.2 mm. The forest stand volume is 75.5 m^3/hm^2. The leaf area index is 6.34. The biomass of tree layer, shrub layer, and herbaceous layer are 112.96, 3.56, and 8.28 t/hm^2, respectively, The plant diversity index of the community is 0.51 (calculated using the Simpson method). Shrubs are mainly *Elaeagnus pungens* Thunb, *Rosa xanthina* Lindl, *Spiraea Salicifolia* L, *Lonicera japonica* Thunb, and *Viburnum dilatatum* Thunb. The shrub coverage is 30%. Herbs are mainly *Carex lanceolata* Boott, and the herb coverage is 30%–50%.

Experimental design

In China, the N deposition rate in 2010 was ranged from 0.1 g N^{-2} y^{-1} to 7.43 g N^{-2} y^{-1}, with an average of 2.11 g N^{-2} y^{-1}, and N deposition is increasing at an annual rate of 0.041 g N^{-2} y^{-1} (Lü and Tian 2007). Thus, we designed four levels of N addition treatments (0, 3, 6, and 9 g N m^{-2} y^{-1}) in the form of urea (Fumin Agriculture Product Company, Xi'an, China). In each treatment, six 10 m × 10 m plots were established for N addition. A total of 24 pots were subjected to the same climate, stand age, and terrain conditions. In 2014, urea was dissolved in 10 L distilled water and evenly sprayed in each plot before the rain in early April, June, August, and October every year. Soil samples were collected in September 2015, and N was added for 2 years.

Soil sampling

In September 2015, undisturbed soil was collected from 0–20 cm soil layer because the short-term N addition directly affected the physical and chemical properties of the surface soil. First, three pits were dug in the central rows of each plot, and undisturbed soil was collected in each pit after removing the surface litter. Second, the undisturbed soil samples were carefully transported back to the laboratory. The undisturbed soils were drilled gently along the natural fissures, and all the soil samples were air-dried after filtering through 8 mm sieve. The three soil samples of each pot were mixed up after air-drying. Finally, all soil samples were stored until physical and chemical analysis.

Aggregate distribution

Soil aggregates were classified by wet sieving method (Six et al. 2010). Air-dried soil sample (100 g) was soaked with deionized water for 5 min under a 250 μm sieve, and the surface of the water submerged the soil samples. Aggregates were separated by moving the sieve up and down for 3 cm with 50 repetitions/min for 2 min. Aggregates retained in the sieve (>250 μm) were macro-aggregates. The soil and water passed through the 250 μm sieve and then were sieved again as above using a 53 μm sieve for 3 min. Aggregates retained in the sieve (>53 μm) were micro-aggregates, and those that passed through the sieve were clay–silt aggregates. All separated soil and water were collected, oven-dried at 45 °C, and weighed and stored for chemical analysis.

GRSP analyses

GRSP content was determined according to the method of Wright and Upadhyaya (1998) (Wright and Upadhyaya 1998). T-GRSP was extracted from soil sample (1 g) with 8 mL of 50 mmol/L citrate solution at pH 7.0 and then autoclaved at 121 °C for 60 min. The supernatant was removed after centrifugation at 10,000 × g for 10 min. This procedure was repeated 5 times on the same sample until the solution was straw-colored. The supernatant was pooled together and stored at 4 °C. EE-GRSP was extracted from soil sample (1 g) with 8 mL of 20 mmol/L citrate solution at pH 7.0 and autoclaved at 121 °C for 30 min. The supernatant was removed after centrifugation at 10,000 × g for 10 min and stored at 4 °C. The T-GRSP or EE-GRSP in the supernatant was determined by Bradford analysis spectrophotometrically at 590 nm light (Wright et al. 1996, Rillig 2004) (Wright and Upadhyaya,1996; Rillig, 2004) .

SOC analyses

SOC content was measured by dichromate oxidation procedure (Nelson and Sommers 1982).

Statistical analyses

Aggregate stability was evaluated by MWD mm and GMD mm. MWD is a comprehensive index for the evaluation of aggregate stability, and GMD is an index of the main grain size distribution of aggregates. The larger value of MWD and GMD indicate higher aggregate stability (Bedini et al. 2009). MWD and GMD were calculated using the following equation:

$$\mathrm{MWD} = \sum_{i=1}^{n} x_i w_i \text{ and } \mathrm{GMD} = EXP\left[\frac{\sum_{i=1}^{n} w_i ln x_i}{\sum_{i=1}^{n} x_i}\right]$$

where x_i is the mean diameter of each class aggregate (mm), w_i is the percentage of each class aggregate (%), and n is the number of aggregate fraction classes.

Differences between any two N addition treatments in aggregate percentage, MWD, GMD, GRSP content, and SOC content were tested with one-way ANOVA on SPSS 20.0 statistical software package (SPSS, Inc., USA). Linear regression analyses were used to test the relationships among GRSP, SOC, and MWD.

Results

Effects of N addition on soil aggregate distribution and stability

The percentage of aggregate varied with size classes, and macro-aggregates were more dominant than the other size aggregate fractions (Table. 1). Across all N addition treatments, the values of MWD were in the range of 1.38–1.70 mm, and GMD was in the range of 0.86–1.05 mm. N addition had no significant effects on aggregate distribution, MWD, and GMD. However, the percentage of macro-aggregate, MWD, and GMD increased first and then decreased with increasing N addition levels, whereas the percentage of micro-aggregate and clay–silt aggregate decreased first and then increased with increasing N addition levels.

Effects of N addition on GRSP content

Figure 1 shows that the contents of EE-GRSP and T-GRSP were in the range of 0.099–0.551 mg/g and 0.315–4.058 mg/g, respectively. In the control treatment, GRSP content varied among bulk soil and aggregate fractions, and GRSP content increased in the order of macro-aggregate<bulk soil<micro-aggregate<clay–silt aggregate. Across all N addition treatments, EE-GRSP content in the macro-aggregates and bulk soil increased first and then decreased with the increasing N addition levels and had a top value at 6 g N^{-2} y^{-1}. Compared with that of control treatment, N addition changed the EE-GRSP content of the macro-, micro-, clay–silt aggregates and bulk soil by –24.8%–222.1%, –30.8%–22.0%, 22.8%–40%, and –8.4%–134.4%. Across all N addition treatments, T-GRSP contents in all size aggregates and bulk soil increased with the increasing N addition levels and had a top value at 9 g N^{-2} y^{-1}. Compared with that of control treatment, N addition changed the T-GRSP content in macro-, micro-, clay–silt aggregates, and bulk soil by 412.7%–872.4%, 203.1%–266.9%, –0.3%–14.3%, and 259.3%–468.0%.

Effects of N addition on SOC content

SOC content ranged from 6.478 mg/g to 21.627 mg/g. Across all aggregate fractions, SOC increased in the following order: clay–silt aggregate < micro-aggregate < macro-aggregate (Fig. 2). Compared with that of control treatment, N addition significantly increased SOC content in all size aggregate fractions and bulk soil, and the change ranges of SOC content in the macro-, micro-, clay–silt aggregates, and bulk soil were 71.7%–79.5%, 75.5%–91.5%, 49.6%–53.5%, and 47.6%–74.4%.

Relationships among GRSP, SOC, and MWD

Table 2 shows that the contents of SOC, EE-GRSP, and T-GRSP in aggregate fractions and bulk soil have no significant relationship with MWD (except for the relationship between SOC content in micro-aggregates and MWD).

Discussion

In this study, the soil of *P. tabulaeformis* forest was dominated by macro-aggregates, and the aggregate stability indexes of MWD and GMD were in the range of 1.38–1.70 mm and 0.86–1.05 mm, respectively. N addition for 2 years had no significant effects on the soil aggregate stability indexes, which was consistent with those of the previous studies (Wang et al. 2013, Zhong et al. 2017). For example, Zhong found that N addition for one year insignificantly changed the soil aggregate distribution in subtropical forest. Although N addition had no significant effect on aggregate distribution and stability, the percentage of macro-aggregate and values of MWD and GMD were higher in N addition treatments compared with that in control treatment. Meanwhile, N addition changed the contents of GRSP and SOC in aggregate fractions, which indicated that N addition could have a trend to change the soil aggregate distribution and could increase the aggregate stability with N addition duration.

Effects of N addition on GRSP content

The contents of EE-GRSP and T-GRSP were in the range of 0.099–0.551 mg/g and 0.315–4.058 mg/g, respectively. This finding is consistent with that of the studied on the GRSP contents of different vegetation communities (0.6–5.8 mg/g) (Singh, Singh and Tripathi 2013). In this study, GRSP content varied among aggregate fractions and bulk soil, and N addition had different effects on GRSP distribution in aggregate fractions and bulk soil. The research in farmland system found that tillage management and manure amendment had different effects on GRSP distribution in aggregate fractions (Wright et al. 2007, Xie et al. 2015). Long-term manure amendments redistributed the GRSP in the macro-aggregate fractions. These results indicated that GRSP distribution in aggregate fractions is susceptible to environmental changes. EE-GRSP was produced by AMF most recently, and EE-GRSP was proved to be more sensitive to environmental changes than T-GRSP (Singh et al. 2013). In our study, the content of EE-GRSP in bulk soil increased first and then decreased with the increasing N addition levels and reached the peak (0.403 mg/g) at 6 g N m^{-2} y^{-1}. The variation in EE-GRSP content was mainly attributed to the changes in plant growth and soil microbial activities (Xie et al. 2015), which were induced by N addition. In our study, plant growth and soil microbial activities might be increased at 3 g N m^{-2} y^{-1} and inhibited at 9 g N m^{-2} y^{-1}. Thus, N addition of 6 g N m^{-2} y^{-1} might be the threshold value between increased and inhibited GRSP production. Xie (Xie et al. 2015) found that the highest EE-GRSP contents were not obtained at the highest amount of organic manure. Our results indicated that low-level N addition could increase the EE-GRSP contents, but high-level N addition inhibited the EE-GRSP contents. The highest EE-GRSP contents were observed at 6 g N m^{-2} y^{-1}, which indicated a proper N addition level can maximize the EE-GRSP content in this region. These results supported our first hypothesis. Furthermore, N addition had different effects on EE-GRSP content in aggregate fractions. The EE-GRSP content in macro-aggregates increased initially and then decreased with the increasing N addition levels and had a peak value at 6 g N m^{-2} y^{-1}. Meanwhile, the EE-GRSP content in micro-aggregates had a low value at 6 g N m^{-2} y^{-1}. These results were inconsistent with our first hypothesis. Previous studies showed that organic manure significantly increased the EE-GRSP content in all soil aggregate fractions except micro-aggregates in farmland ecosystems (Xie et al. 2015), and increasing soil depth significantly increased the EE-GRSP content in 0.5–1.0 and 1–2 mm aggregates without other size aggregate fractions in citrus rhizosphere (Wu et al. 2013). These different results indicated that the distribution of EE-GRSP in aggregate fractions varied with environmental conditions, which resulted in the changes of EE-GRSP in bulk soil. In addition, the percentage of macro-aggregates was higher than those of other size aggregate fractions. N addition has the same effects at EE-GRSP content in macro-aggregates with that in bulk soil, which indicated that the EE-GRSP in macro-aggregates was the main factor that determined the EE-GRSP in bulk soil. T-GRSP contained easily and hardly extractable parts. Compared with EE-GRSP, T-GRSP can reflect the accumulation of glomalin in the soil (Singh et al. 2013). In our study, N addition significantly increased the T-GRSP content in bulk soil and had a peak value at 9 g N m^{-2} y^{-1}, which was different from the changes of EE-GRSP content in bulk soil. A large number of studies found that environmental changes had different effects on T-GRSP and EE-GRSP contents in bulk soil as well (Wright et al. 2007, Xie et al. 2015, Antibus et al. 2006, Zhang et al. 2015). A explanation for this result in our study is that high because high-level N addition can quickly relieve N limitation in the soil and promote the microbial activities at the beginning of the experiment, which increase GRSP production in the short term. Given the increasing time, high-level N addition may cause N saturation in the soil and inhibit microbial activities that decrease the GRSP production (Yu et al. 2013). However, the T-GRSP content under high N addition level was higher than that under low N addition level for a period of time due to the cumulative effect. Furthermore, the T-GRSP content in aggregate fractions increased significantly with the N addition, and this result supports our explanation.

Effects of N addition on SOC content

In this study, SOC content ranged from 6.478 and 21.627 mg/g. Across all aggregate fractions, macro-aggregate had the highest SOC content, followed by the micro-aggregate, and those in clay-silt aggregate is the lowest. These results were consistent with that of the previous study (Zhang et al. 2014). However, some studies showed that the highest SOC content was in clay–silt aggregate, and the lowest was in macro-aggregate (Gao et al. 2013, Tang et al. 2016). Different results might be caused by soil type, plant community, and experimental conditions (Wei et al. 2011). N addition for 2 years significantly increased SOC content in bulk soil, which indicated that N addition could quickly increase plant growth in N limitation soil and increase soil C storage (Nave et al. 2009). Interestingly, N addition significantly increased SOC content in all size aggregate fractions. Compared with other size aggregate fractions, macro-aggregate had the highest percentage and SOC content, with the greatest contribution to the soil C storage. Meanwhile, the SOC in clay–silt aggregate had the smallest contribution to the soil C storage. These results were different from our hypothesis. Zhong (Zhong et al. 2017) found that N addition (0–300 kg N ha^{-1} y^{-1}) for 1 year had no significant influence on SOC in any size aggregate fractions in a subtropical forest. These different results might be related to soil type and initial N content. For example, macro- and micro-aggregate fractions had the highest SOC content among the size aggregate fractions in loamy soils, while clay–silt aggregate fraction had the highest SOC content than those of other size aggregate fractions in sandy soil (Gao et al. 2013). Our results indicated that the SOC content varied among aggregate fractions, and N addition had different effects on SOC in aggregate fractions. The effects were not only affected by N addition level but also by soil type and initial N content.

Relationships among GRSP, SOC, and aggregate stability

GRSP and SOC were insignificantly correlated with MWD. This result fails support our hypothesis. A large number of studies found that GRSP and SOC were significantly correlated with MWD because both can bind soil particle for aggregate formation (Rillig 2004, Wright et al. 2007, Baldock, Kay and Schnitzer 1987). However, recent studies found that GRSP and/or SOC may have no significant relationship with MWD (Wu et al. 2013, Li et al. 2005, Leelamanie and Mapa 2015) because aggregate stability was not only affected by GRSP and SOC contents but also by soil texture, human disturbance, and other factors (Wei et al. 2011, Tang et al. 2016). In our study, the contents of GRSP and SOC significantly changed, but MWD insignificantly changed with N addition levels which caused insignificant relationships among MWD, GRSP, and SOC. N addition significantly increased the contents of GRSP and SOC and insignificantly increased MWD, indicating that increasing GRSP and SOC contents does not necessarily increase aggregate stability rapidly. Aggregate formation requires not only binding agents but also physicochemical effects, but the underlying mechanism still needs further study.

Conclusions

N addition has different effects on GRSP content in aggregate fractions at *P. tabulaeformis* forestland. The EE-GRSP content in macro-aggregates increased initially and then decreased with the increasing N addition levels and had a peak value at 6 g N m^{-2} y^{-1}, indicating a proper N addition level might maximize the GRSP production in this region. The T-GRSP content in aggregate fractions increased significantly with the N addition and had a peak value at 9 g N m^{-2} y^{-1}, because of the cumulative effect. Compare with control treatment, SOC content in all size aggregate fractions significantly increased in N addition treatments, which indicate that N addition could quickly increase plant growth in N limitation soil and increase soil C storage. However, N addition for 2 years has no significant effects on MWD and GMD, and GRSP and SOC are insignificantly correlated with MWD. This study identifies that the underlying mechanism of aggregate formation requires binding agents and reaction time both. Increasing GRSP and SOC contents does not necessarily increase aggregate stability rapidly.

References

Antibus, R. K., C. Lauber, R. L. Sinsabaugh & D. R. Zak (2006) Responses of Bradford-reactive soil protein to experimental nitrogen addition in three forest communities in northern lower Michigan. *Plant & Soil,* 288**,** 173-187.

Bai, Y., W. U. Jianguo, C. M. Clark, S. Naeem, Q. Pan, J. Huang, L. Zhang & G. Xing (2010) Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. *Global Change Biology,* 16**,** 358-372.

Baldock, J. A., B. D. Kay & M. Schnitzer (1987) Influence of cropping treatments on the monosaccharide content of the hydrolysates of a soil and its aggregate fractions. *Canadian Journal of Soil Science,* 67**,** 489-499.

Bedini, S., E. Pellegrino, L. Avio, S. Pellegrini, P. Bazzoffi, E. Argese & M. Giovannetti (2009) Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species Glomus mosseae and Glomus intraradices. *Soil Biology & Biochemistry,* 41**,** 1491-1496.

BlancoCanqui, H. & R. Lal (2004) Mechanisms of Carbon Sequestration in Soil Aggregates. *Critical Reviews in Plant Sciences,* 23**,** 481-504.

Chen, Z. J., S. C. Geng, J. H. Zhang, H. Setälä, Y. Gu, F. Wang, X. Zhang, X. X. Wang & S. J. Han (2017) Addition of nitrogen enhances stability of soil organic matter in a temperate forest. *European Journal of Soil Science,* 68.

Das, B., D. Chakraborty, V. K. Singh, P. Aggarwal, R. Singh & B. S. Dwivedi (2014) Effect of

Organic Inputs on Strength and Stability of Soil Aggregates Under Rice-Wheat Rotation. *International Agrophysics,* 28**,** 163-168.

Fonte, S. J., E. Yeboah, P. Ofori, G. W. Quansah, B. Vanlauwe & J. Six (2009) Fertilizer and residue quality effects on organic matter stabilization in soil aggregates. *Soil Science Society of America Journal,* 73**,** 961-966.

Galloway, J. N., F. J. Dentener, D. G. Capone, E. W. Boyer, R. W. Howarth, S. P. Seitzinger, G. P. Asner, C. C. Cleveland, P. A. Green & E. A. Holland (2004) Nitrogen Cycles: Past, Present, and Future. *Biogeochemistry,* 70**,** 153-226.

Gao, H., L. Qiu, Y. Zhang, L. Wang, X. Zhang & J. Cheng (2013) Distribution of organic carbon and nitrogen in soil aggregates of aspen (Populus simonii Carr.) woodlands in the semi-arid Loess Plateau of China. *Soil Research,* 51**,** 406.

Garcia, M. O., T. Ovasapyan, M. Greas & K. K. Treseder (2008) Mycorrhizal dynamics under elevated CO 2 and nitrogen fertilization in a warm temperate forest. *Plant & Soil,* 303**,** 301-310.

Kathleenk, T., T. Katiem & M. Michellec (2007) Mycorrhizal responses to nitrogen fertilization in boreal ecosystems: potential consequences for soil carbon storage. *Global Change Biology,* 13**,** 78-88.

Keeler, B. L., S. E. Hobbie & L. E. Kellogg (2009) Effects of Long-Term Nitrogen Addition on Microbial Enzyme Activity in Eight Forested and Grassland Sites: Implications for Litter and Soil Organic Matter Decomposition. *Ecosystems,* 12**,** 1-15.

KH, B., B. EM, D. RA, H. KS, J. ES, S. JE & C. MJ (2014) A long-term nitrogen fertilizer gradient has little effect on soil organic matter in a high-intensity maize production system. *Global Change Biology,* 20**,** 1339.

Lü, C. & H. Tian (2007) Spatial and temporal patterns of nitrogen deposition in China: Synthesis of observational data. *Journal of Geophysical Research Atmospheres,* 112**,** 229-238.

Leelamanie, D. A. L. & R. B. Mapa (2015) Alterations in soil aggregate stability of a tropical Ultisol as mediated by changes in land use. *Biologia,* 70**,** 1444-1449.

Li, Z. X., C. F. Cai, Z. H. Shi & T. W. Wang (2005) Aggregate stability and its relationship with some chemical properties of red soils in subtropical China. *Pedosphere,* 15**,** 129-136.

Liu, X., G. Yin, J. Wu, J. Chen, Z. He & S. Shi (2015) Effect of organic carbon and total nitrogen distribution in alpine meadow soil aggregates with different nitrogen addition level. *Nongye Gongcheng Xuebao/transactions of the Chinese Society of Agricultural Engineering,* 31**,** 139-147.

Nave, L. E., E. D. Vance, C. W. Swanston & P. S. Curtis (2009) Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. *Geoderma,* 153**,** 231-240.

Nelson, D. W. & L. E. Sommers (1982) Total carbon, organic carbon and organic matter, in: Methods of Soil Analysis Part 2. Chemical and Microbial Properties.

Pan, G. X., P. Zhou, L. Q. Li & X. H. Zhang (2007) Core issues and research progresses of soil science of C sequestration. *Acta Pedologica Sinica*.

Rillig, M. C. (2004) Arbuscular mycorrhizae, glomalin, and soil aggregation. *Canadian Journal of Soil Science,* 84**,** 355-363.

Singh, P. K., M. Singh & B. N. Tripathi (2013) Glomalin: an arbuscular mycorrhizal fungal soil protein. *Protoplasma,* 250**,** 663-669.

Six, J., E. T. Elliott, K. Paustian & J. W. Doran (2010) Aggregation and Soil Organic Matter Accumulation in Cultivated and Native Grassland Soils. *Soil Science Society of America Journal,* 62**,** 1367-1377.

Tang, F. K., M. Cui, Q. Lu, Y. G. Liu, H. Y. Guo & J. X. Zhou (2016) Effects of vegetation restoration on the aggregate stability and distribution of aggregate-associated organic carbon in a typical karst gorge region. *Solid Earth,* 7**,** 141-151.

Treseder, K. K. (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO 2 in field studies. *New Phytologist,* 164**,** 347-355.

Treseder, K. K. & M. F. Allen (2002) Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. *New Phytologist,* 155**,** 507-515.

Tripathi, S. K., C. P. Kushwaha & K. P. Singh (2008) Tropical forest and savanna ecosystems show differential impact of N and P additions on soil organic matter and aggregate structure. *Global Change Biology,* 14**,** 2572–2581.

Wang, F., Y. A. Tong, J. S. Zhang, P. C. Gao & J. N. Coffie (2013) Effects of various organic materials on soil aggregate stability and soil microbiological properties on the Loess Plateau of China. *Plant Soil & Environment,* 59**,** 162-168.

Wang, G. L., S. Xue, F. Liu & G. B. Liu (2017) Nitrogen addition increases the production and turnover of the lower-order roots but not of the higher-order roots of Bothriochloa ischaemum. *Plant and Soil***,** 423–434.

Wei, Y. W., Y. R. Su, X. B. Chen, X. Y. He, W. G. Qin & G. F. Wei (2011) [Effects of human disturbance on soil aggregates content and their organic C stability in Karst regions]. *Ying Yong Sheng Tai Xue Bao.,* 22**,** 971-978.

Wright, F. Sara, Upadhyaya & Abha (1996) EXTRACTION OF AN ABUNDANT AND UNUSUAL PROTEIN FROM SOIL AND COMPARISON WITH HYPHAL PROTEIN OF ARBUSCULAR MYCORRHIZAL FUNGI. *Soil Science,* 161**,** 575-586.

Wright, S. F., V. S. Green & M. A. Cavigelli (2007) Glomalin in aggregate size classes from three different farming systems. *Soil & Tillage Research,* 94**,** 546-549.

Wright, S. F. & A. Upadhyaya (1998) A survey of soils for aggregate stability and glomalin a glycoprotein of arbuscular mycorrhizal fungi. Plant Soil 198: 97-107. *Plant & Soil,* 198.

Wu, Q. S., X. H. He, M. Q. Cao, Y. N. Zou, W. Shuang & L. Yan (2013) Relationships between glomalin-related soil protein in water-stable aggregate fractions and aggregate stability in citrus rhizosphere. *International Journal of Agriculture & Biology,* 15**,** 603-606.

Wuest, S. B., T. C. Caesar-Tonthat, S. F. Wright & J. D. Williams (2005) Organic matter addition, N, and residue burning effects on infiltration, biological, and physical properties of an intensively tilled silt-loam soil. *Soil & Tillage Research,* 84**,** 154-167.

Xie, H., J. Li, B. Zhang, L. Wang, J. Wang, H. He & X. Zhang (2015) Long-term manure amendments reduced soil aggregate stability via redistribution of the glomalin-related soil protein in macroaggregates. *Scientific Reports,* 5**,** 14687.

Yin, J., R. Wang, H. Liu, X. Feng, Z. Xu & Y. Jiang (2016) Nitrogen addition alters elemental stoichiometry within soil aggregates in a temperate steppe. *Solid Earth Discussions,* 7**,** 1565-1575.

Yu, P. Y., F. Zhu, S. F. Su, Z. Y. Wang & W. D. Yan (2013) [Effects of nitrogen addition on red soil microbes in the Cinnamomum camphora plantation]. *Environmental Science,* 34**,** 3231.

Zak, D. R. & R. L. Sinsabaugh (2004) Nitrogen Deposition Modifies Soil Carbon Storage through Changes in Microbial Enzymatic Activity. *Ecological Applications,* 14**,** 1172-1177.

Zhang, J., X. Tang, X. He & J. Liu (2015) Glomalin-related soil protein responses to elevated CO 2 and nitrogen addition in a subtropical forest: Potential consequences for soil carbon accumulation. *Soil Biology & Biochemistry,* 83**,** 142-149.

Zhang, X., X. Wu, S. Zhang, Y. Xing, R. Wang & W. Liang (2014) Organic amendment effects on aggregate-associated organic C, microbial biomass C and glomalin in agricultural soils. *Catena,* 123**,** 188-194.

Zhong, X. L., J. T. Li, X. J. Li, Y. C. Ye, S. S. Liu, P. D. Hallett, M. R. Ogden & M. Naveed (2017) Physical protection by soil aggregates stabilizes soil organic carbon under simulated N deposition in a subtropical forest of China. *Geoderma,* 285**,** 323-332.

Table 1(on next page)

The soil aggregate characteristic of different N addition treatments

values followed by a different letter indicate significant difference among the treatments. MWD: Mean weight diameter, GMD： Geometric mean diameter. ($P<0.05$ n=6).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26649v1 | CC BY 4.0 Open Access | rec: 9 Mar 2018, publ: 9 Mar 2018

Table 1 The soil aggregate characteristic of different N addition treatments

Treatment	Aggregate proportion in size class (%)			MWD(mm)	GMD(mm)
(g N m^{-2} y^{-1})	>0.25mm	0.053-0.25mm	<0.053mm		
CK(0)	65.36±7.63a	28.49±6.40a	6.16±1.98a	1.45±0.073a	0.90±0.039a
N3(3)	68.78±7.99a	25.95±7.41a	5.28±0.72a	1.50±0.158a	0.94±0.086a
N6(6)	73.65±5.05a	21.88±4.57a	4.48±0.90a	1.60±0.101a	0.99±0.063a
N9(9)	72.76±7.82a	22.11±6.45a	5.14±1.48a	1.58±0.156a	0.98±0.099a

Note: values followed by a different letter indicate significant difference among the treatments. MWD: Mean weight diameter, GMD：Geometric mean diameter. ($P<0.05$ n=6).

Table 2(on next page)

Bivariate correlations (r values) between the mean weight diameter (MWD) and soil organic carbon (SOC), easily extractable and total glomalin-related soil protein (EE-GRSP and T-GRSP).

asterisk indicates a significant correlation, ($P < 0.05$. n=24).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26649v1 | CC BY 4.0 Open Access | rec: 9 Mar 2018, publ: 9 Mar 2018

Table 2 Bivariate correlations (r values) between the mean weight diameter (MWD) and soil organic carbon (SOC), easily extractable and total glomalin-related soil protein (EE-GRSP and T-GRSP).

	SOC	EE-GRSP	T-GRSP
Bulk soil	0.381	0.176	0.250
>0.25mm	0.254	0.156	0.266
0.25-0.053mm	0.420*	-0.050	0.318
<0.053mm	0.376	0.098	-0.006

asterisk indicates a significant correlation, ($P < 0.05$. n=24).

Figure 1(on next page)

Distributions of easily extractable and total glomalin-related soil protein (A: EE-GRSP and B: T-GRSP) concentration in soil aggregates and bulk soil of different N addition treatments.

Values followed by a different lowercase letters indicate significant difference among the N addition treatments, values followed by a different uppercase letters indicate significant difference among the aggregate size. ($P<0.05$ $n=6$).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26649v1 | CC BY 4.0 Open Access | rec: 9 Mar 2018, publ: 9 Mar 2018

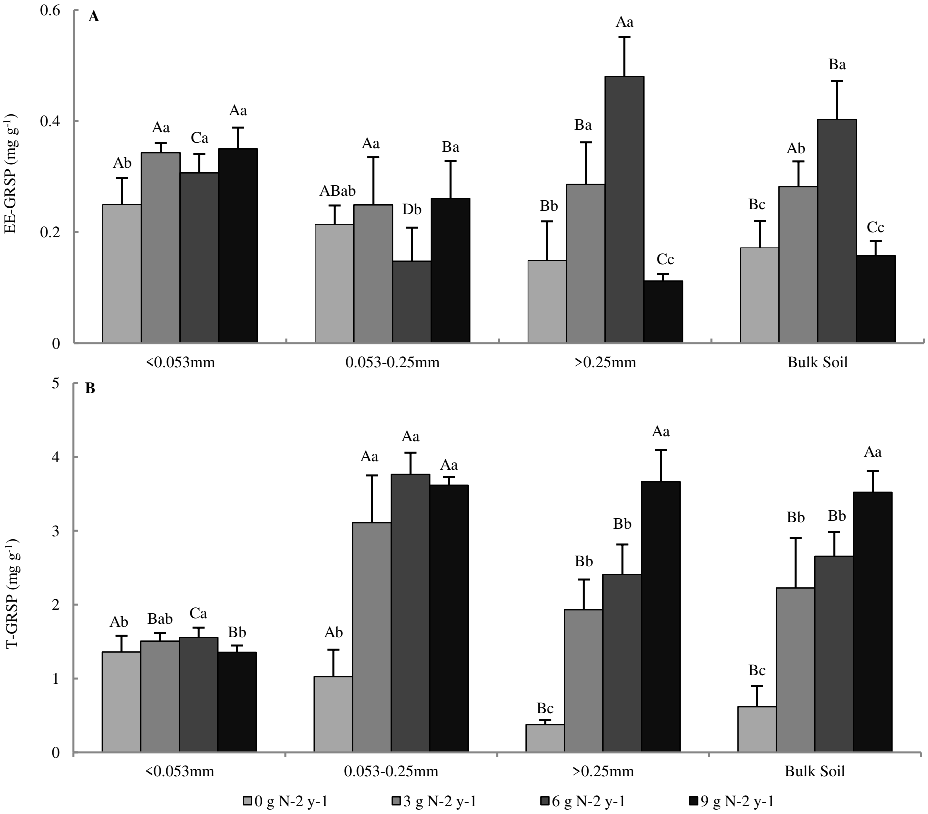

Figure 1 Distributions of easily extractable and total glomalin-related soil protein (A: EE-GRSP and B: T-GRSP) concentration in soil aggregates and bulk soil of different N addition treatments

Note: Values followed by a different lowercase letters indicate significant difference among the N addition treatments, values followed by a different uppercase letters indicate significant difference among the aggregate size. (*P*<0.05 n=6).

Figure 2(on next page)

Distributions of organic C concentration in soil aggregates and bulk soil of different N addition treatments.

Values followed by a different lowercase letters indicate significant difference among the N addition treatments, values followed by a different uppercase letters indicate significant difference among the aggregate size. (P<0.05 n=6).

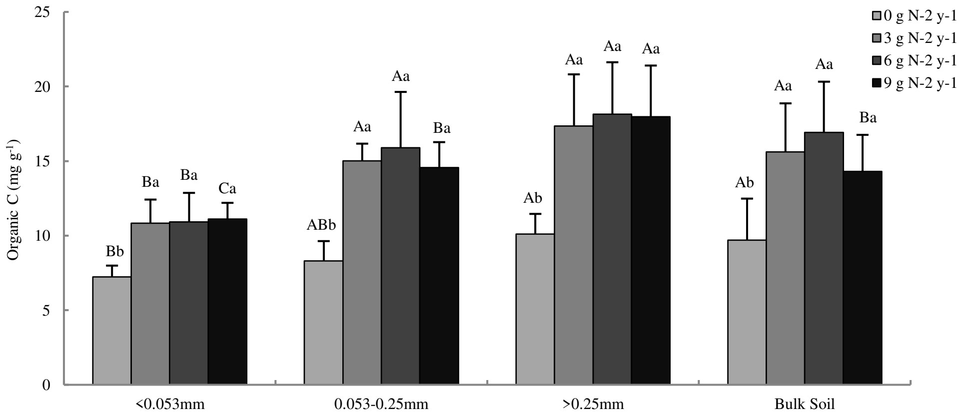

Figure 2 Distributions of organic C concentration in soil aggregates and bulk soil of different N addition treatments

Note: Values followed by a different lowercase letters indicate significant difference among the N addition treatments, values followed by a different uppercase letters indicate significant difference among the aggregate size. (*P*<0.05 n=6).

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26649v1 | CC BY 4.0 Open Access | rec: 9 Mar 2018, publ: 9 Mar 2018