Long-Term Fertilization Alters the Chemical Composition of Dissolved Organic Carbon via Regulating Soil Physicochemical Factors

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Abstract

Soil dissolved organic carbon (DOC) plays a key role in fundamental biogeochemical processes; however, the influences of different types of fertilization on the chemical composition and properties of DOC molecules in soils are poorly understood. In this study, DOC samples were extracted from gray desert soils treated with five fertilization treatments 1) chemical nitrogen, phosphorus and potassium fertilizers; 2) pig and cattle manure; 3) 50% nitrogen from manure and 50% nitrogen from chemical fertilizer; 4) total chemical fertilizers and total manure; and 5) no fertilization (control). Data were compared by a combination of Fourier-transform infrared spectroscopy, ^{13}C nuclear magnetic resonance, and statistical analyses. Results showed that manure application increased the aliphatic carbon and decreased the aromatic carbon levels, implying manure application promotes the transformation of labile carbon to stable carbon structures. Redundancy analysis indicated that available nutrients and available forms of magnesium were positively associated with the labile carbon groups, and the available forms of calcium were positively associated with the stable carbon groups. These results demonstrated that the availability of soil nutrients and minerals are influential factors on DOC turnover. Moreover, our results showed that fertilization method had negative direct effects on DOC transformation and positive indirect effects on DOC turnover via soil physicochemical factors, and various forms of mineral ions were the strongest explanatory factors of DOC variation. These findings indicated that soil physicochemical factors play an important role as mediators in the influence of fertilization practices on the chemical composition of DOC and turnover of carbon-containing functional groups in DOC.

1. Introduction

Dissolved organic carbon (DOC), a small and labile forms of soil organic carbon, plays an important role in various biogeochemical processes (Taylor and Townsend 2010). Its composition directly provides information on the production or degradation of organic matter (Schmidt et al. 2011). It primarily originates microbial metabolites, plant biomass and organic matter (Malik and Gleixner 2013; Roth et al. 2019). Its production and turnover are affected by biotic factors (e.g., microbial diversity and enzyme activity) and abiotic factors (e.g., moisture and temperature). Among all factors, the forms of soil clay minerals appear to be one of most significant factors because they affect the decomposition of soil organic carbon by the physical and chemical protection (Yu et al. 2017). For example, exchangeable mineral ions preferentially bind organic acid molecules that are highly unsaturated or rich in oxygen by ionic or covalent bonds (Yu et al. 2012). The latest information about DOC have shown that various forms of soil mineral ions affect the transformation of DOC by selectively retaining the DOC carbon groups (e.g., carboxylate bound to the surface of calcium carbonate) or by regulating the abundances of microorganisms that affect DOC turnover (Leinemann et al. 2018; Wen et al. 2014).

Soil aggregates influences the DOC composition and turnover by mediating the kinetics processes of soil organic matter (Xu et al. 2021). Mineral-bound organic molecules can be retransformed through the destruction or construction of soil aggregates (Zhao et al. 2020). Aggregate stabilization processes (e.g., cementation and agglomeration) can alter remobilized DOC molecules or retransformation of carbon-

containing groups by spatial isolation (Xu et al. 2019). Those changes in DOC are accompanied by variations in soil physicochemical properties. Soil physicochemical properties (e.g., soil nutrient availability, pH and cation exchange capacity) not only influence the molecular properties of DOC (Smebye et al. 2016) but also affect the efficiency of the microbial metabolism in degrading carbon-containing groups (Chen et al. 2017). The microbial community probably assembles new molecules or groups in the consumption and degradation of DOC (Wu et al. 2018).

Besides biotic factors and abiotic factors, the agronomic practices such as tillage (Bongiorno et al. 2019), irrigation (Said-Pullicino et al. 2016) and fertilization (Li et al. 2019) also influence DOC production and turnover. Fertilization is one of the primary factors influencing DOC turnover in agronomic practices. Chemical nitrogen fertilizer application promotes the preferential mineralization of saturated carbon groups (e.g., alkanes) and accumulation of unsaturated carbon (e.g., aromatic groups) in DOC by changing the ratio of carbon to nitrogen (C/N) in soils (Wang et al. 2020). Meanwhile, a long-term fertilization experiment indicates that aromatic carbon content increases with nitrogen addition (Li et al. 2019). The stepwise degradation of cellulose to extended aromatic compounds, an important contributor to DOC production, has shown to be more efficient in nitrogen-fertilized than non-fertilized plots (Yuan et al. 2018).

The current understanding of fertilization on DOC turnover suggests that fertilization directly influence the amount or ratio of labile group and inert group in DOC by the inherent inputs of exogenous matter (Singh et al. 2014), or indirectly impact DOC composition by regulating microbial activity and community (Wang et al. 2015; Zang et al. 2017). However, recent results reveal that DOC composition and turnover may be more susceptible to the influential factors of its surrounding environment (e.g., nutrient availability, aggregate stability and mineral forms) than to fertilization (Li et al. 2019; Wang et al. 2021). Furthermore, one study found that long-term fertilization influences DOC composition mainly via regulating soil aggregate size, not direct effect (Xu et al. 2021).

Gray desert soil, developed from loess parent material, is an important zonal soil in temperate desert areas with rich in the minerals of calcium and magnesium (Wang et al. 2016). The area where it is located has less precipitation and a high rate of DOC production and turnover. The increased mineralization of organic matter caused by the rapid turnover of DOC has become one of the main obstacles to the development of local agriculture and animal husbandry (Wang et al. 2016). The relationships between DOC properties and minerals of calcium or magnesium have been reported (Minick et al. 2017; Zhu et al. 2019), but the underlying mechanisms of how mineral forms of calcium or magnesium act as mediators to regulate the DOC turnover are still largely unknown.

Applying the Fourier transform infrared (FTIR) spectroscopy and ^{13}C nuclear magnetic resonance (^{13}C -NMR) techniques has allowed us to capture the variation in carbon-containing groups of DOC (Hayes et al. 2017). This provides a more detailed molecular-level understanding of DOC-group turnover. In this study, we conducted a long-term field experiment comparing different fertilization managements and used FTIR spectroscopy and ^{13}C -NMR to quantitatively determine how the different fertilization strategies

affected the composition and chemical properties of DOC. We hypothesized that soil physicochemical properties are the key agents for the DOC turnover driven by fertilization practices. We characterized the chemical composition of DOC and analyzed the implications of fertilization on the turnover of DOC groups and the role of soil physical and chemical properties in the turnover process. We expect that the results will contribute to understanding the DOC turnover and help to predict carbon cycling in agro-ecosystems.

2. Materials And Methods

2.1. Field experiment and sampling

The field experiment is located near Hexi Corridor in Gansu Province, Northwest China (37°53' N, 102°40' E, elevation 1540 m; MAP and MAT of 150 mm and 7.7°C, respectively). We previously reported a detailed description of field site, treatment, soil sampling and analyses (Zhang et al., 2021). In brief, three soil cores (0–20 cm deep) were collected in March 2019 from plots that were established in 2007 with one of five fertilization strategies (chemical nitrogen, phosphorus and potassium fertilizers [NPK], pig and cattle manure [M], 1/2NPK and 1/2M [1/2MNPK], total NPK and total M [MNPK], and no-fertilization control [CT]). Three soils were composited in one soil sample per plot and 500 g of the composite sample was taken as the final sample. All soil samples were air-dried and each passed through a 2-mm sieve prior to further analyses and DOC extraction experiments. The methods of measuring and determining the indices of basic soil properties, aggregate stability, calcium forms and magnesium forms are presented in Supplementary Appendix S1.

2.2. DOC preparation and characterization

In this study, the water-extractable organic carbon was used to instead of the organic carbon in soil solution. Although the water-extractable organic carbon does not exclusively reflect the in-situ dissolved composition, the water-extractable organic carbon contains almost all the carbon groups in the soil solution and does not affect the characterization of dissolved organic carbon composition (Ding et al. 2020). Therefore, the water-extractable organic carbon was considered equivalent to the dissolved organic carbon in soil samples and henceforth is referred to as DOC. The extraction method was provided by Ding et al. (2020). Briefly, soil samples (soil: water = 3: 5) were shaken (24 h at 25°C), centrifuged (4000 r, 30 min) and filtered (0.45 µm polyether sulfone filters). Filtered supernatant samples were stored at 4°C until analysis. A solid phase extraction was used to concentrate the DOC. In brief, the pre-acidified DOC samples (pH = 2) were loaded into solid-phase extraction cartridges (Bond Elut C 18, Certified, PPL, SCX) that were pre-soaked overnight in methanol and rinsed with a solution (pH = 2) of ultrapure water, methanol and hydrochloric acid. After the DOC samples were loaded, the cartridges were rinsed with acidified ultrapure water and dried with nitrogen gas. The methanol was used to elute the DOC extracts. An elemental auto-analyzer (Vario MAX CN, Elementar Analysensysteme GmbH, Germany) was used to determine the concentrations of DOC and DON (dissolved organic nitrogen). A FTIR spectrometer (Nicolet 6700, Thermo Fisher Scientific Inc., USA) and a solid state ¹³C NMR spectrometer (Bruker Avance III HD

400 Spectrometer, Bruker BioSpin, Germany) were used to characterize the composition of carbon-containing groups in the DOC. The detailed information of FTIR and ^{13}C -NMR analyses are presented in Supplementary Appendix SI.

2.3. Data analysis

SPSS 21.0 software (IBM Inc., Armonk, NY, USA) was used to perform the statistical analyses of the data (mean $\pm$ SD, $n = 3$). One-way analysis of variance were used to determine significant differences in the carbon aliphatic and carbon aromaticity indices among fertilization treatments at $P < 0.05$. The Bray – Curtis distances between each treatment were calculated to investigate the general differences in the DOC composition in each fertilization treatment. Considering that repeated measurements were taken from the same plots, linear mixed-effects models were used and data were fitted by the “nlme” package in R (R version 4.0.2) to test whether soil nutrient background effects impact carbon-containing groups turnover. To summarize covariations between soil physicochemical factors (e.g., basic soil properties, aggregate stability, calcium forms and magnesium forms) and the DOC composition, redundancy analysis was performed with the “vegan” package in R (R version 4.0.2). Variation partitioning based on the comparison of variance explained by linear models including every possible combination of variables being proxies for soil physicochemical factors were performed. We conducted intermediary analysis in R (R version 4.0.2) using the “mediation” package to investigate the direct and indirect effects of fertilization practices on DOC turnover. Origin 2020 (OriginLab, Northampton, USA) was used to visualize the results.

3. Results

3.1. Chemical DOC characteristics in different fertilization plots

Among the treatments, DON concentrations were higher with the full than without the full manure application (P -value = 0.008), with the largest difference observed in the M and MNPK treatments from the other three treatments (Table S2). Concentrations of DOC was not affected by manure or chemical fertilizer applications (P -value = 0.456), and consequently the ratio of DOC and DON decreased in the plots with manure application. Across all samples taken from the 15 plots, 7 carbon-containing functional groups (N-alkyl, O-alkyl, alkyl, carbohydrate, phenolic, aryl and carbonyl) of DOC compounds were identified (Fig. 1). Most of the detected functional groups were present in all plots with fertilization, but with varying relative abundances (Fig. 1 and Table S3). In comparison to CT, O-alkyl, phenolic, aryl and carbonyl abundances were significantly higher and N-alkyl and alkyl abundances were significantly lower in the plots with manure application (Fig. 1); the difference was most significant in the MNPK plots (Table S3). Moreover, NMR spectra indicated that the relative proportions of unsaturated groups (phenolic, aryl, and sp^2 -hybridized carbons) were the highest and the amounts of saturated groups (alkyl and carbohydrate, sp^3 -hybridized carbon) were the lowest in the MNPK plots (Fig. 1b). The relative

proportions of carbonyl carbon and O-alkyl carbon were similar among fertilization plots and were significantly greater than that of CT (Fig. 1b).

3.2. Fertilization effects on DOC composition

The carbon aliphatic index number of the M treatment was lower and carbon aromaticity index number of the M treatment was higher than their respective index numbers of CT, reflecting the decline in amount of aliphatic carbon and rise in amount of aromatic carbon in M (Fig. 2). The chemical fertilizer application did not change the indices of aliphatic and aromatic carbon compared to those of CT (Fig. 2). The effects of fertilization practices on the DOC properties occurred via the changes in basic soil properties, aggregate stability and the chemical forms of calcium and magnesium. The labile carbon groups (i.e. N-alkyl carbon, alkyl carbon and carbohydrate carbon) were positively associated with total phosphorus, total potassium, available forms of calcium (free and exchangeable calcium), the stable form of magnesium (residual magnesium), and the stable carbon groups (i.e., phenolic, aryl and carbonyl carbon). The same carbon groups were also positively associated with available nutrients (available nitrogen, phosphorus and potassium), cation exchange capacity, soil organic carbon, available forms of magnesium (free and exchangeable magnesium), and the stable form of calcium (residual calcium, Fig. 3).

To evaluate the direct and indirect effects of fertilization practices on the DOC turnover, we analyzed the pathways of effects of fertilization practices on DOC chemical composition. Fertilization practices had direct negative effects and indirect positive effects (Fig. 4). The direct effect of fertilization practices on carbohydrate, carbonyl and N-alkyl carbons were significantly greater than on alkyl, aryl and phenolic carbons (Fig. 4). The indirect effects of fertilization practices on DOC chemical composition were mainly reflected by the significant fertilization effects on N-alkyl and carbohydrate carbons through soil physicochemical properties and the significant effects on alkyl carbon through calcium forms (Fig. 4). Furthermore, to estimate the relative importance of potential factors on DOC turnover, we compared the influences of soil physiochemical factors (i.e., soil physicochemical properties, calcium forms, magnesium forms and aggregate stability). Variation partitioning showed that the soil physiochemical factors accounted for 51% of the variation in composition of DOC (Fig. 5). Basic soil physicochemical properties were the most important predictor (explaining 21% of the DOC composition variation) followed by calcium forms (17%) and magnesium forms (13%, Fig. 5). In addition, a large part of the variation (11%) was jointly explained by basic soil properties, calcium forms and magnesium forms. However, nearly 30% of the variation remains unexplained (Fig. 5), indicating that other agro-ecological factors contributed to the variability in DOC turnover. The unexplained variability might be related to irrigation, cultivation, crops and soil microorganisms.

4. Discussion

4.1. Fertilization alters DOC chemical composition

Although fertilization has little effect on the concentration of DOC (Table S2), it significantly affected the chemical characteristics of DOC composition. A semi-quantitative approximation of the carbon-containing groups abundances was applied and the ratios of aromatic carbon to aliphatic carbon were determined to evaluate whether DOC turnover driven by fertilization method is a result of the disappearance of the labile carbon-containing groups or of the new formations of stable carbon-containing groups (He et al. 2018; Yeasmin et al. 2017). Therefore, we scaled the relative signal intensities of FTIR and ^{13}C -NMR data to the abundances of DOC groups. Those approximations indicated that the lower carbon aliphatic index value and the higher carbon aromaticity index value from M plots (compared to CT) were caused by the respective decreases in abundance of labile carbon-containing groups (i.e., N-alkyl, alkyl and carbohydrate carbons) and increases in abundance of the stable carbon-containing groups (i.e. phenolic, aryl and carbonyl carbons; Figs. 1 and 2). The increase of stable carbon-containing groups promoted the enrichment of polycyclic aromatics and other highly aromatic compounds (e.g., phenol and benzoic acid), causing a reduction of DOC degradation (Li et al. 2018). Some authors reported that manure addition reduces the degradation of DOC molecules because manure decomposition produces considerable amounts of polycyclic aromatic compounds, resulting in low-molecular-mass oxygenated polycyclic aromatic hydrocarbons (Wen et al. 2021). Although manure application increased the carbon aromaticity of DOC, the chemical fertilizer addition seemed to limit the increase (Fig. 2), suggesting the increase of aromaticity and unsaturation of DOC may be derived from microbial necrosis in addition to decomposition products or microbial residue (Crowther et al. 2015). Unexpectedly, we found that compared to CT, carbon aromaticity of DOC increased in the NPK plots, but the carbon aliphatic index was not significantly lower, indicating chemical fertilizers likely promoted the enrichment of aromatic carbon in DOC not from the turnover of aliphatic carbon in the DOC, but from the other organic carbon forms. Moreover, variation between DOC groups were the most significant in MNPK (Table S3), implying the combined application of manure and chemical fertilization accelerated the internal transformation in labile groups or stable groups, which was similar to the results reported by Wang et al. (2015). These differences of molecular composition and properties of DOC among fertilization treatments furthermore confirm the shift from low-molecular-weight to high-molecular-weight compounds was easily disturbed by agricultural management practices (Seifert et al. 2016). Furthermore, our evidence verifies the disturbance effects mainly concentrated on alkanes and aromaticity (Table S4), which may be explained by the facts that alkanes are the carbon sources of microbial propagation and aromaticity is the product of microbial metabolites (Wang et al. 2020).

4.2. Mediation of effects of soil physiochemical factors on DOC turnover

The likely primary mechanisms of DOC turnover are molecular self-assembly and microbial transformation (Roth et al. 2019). Low-weight molecular compounds are assembled into medium- and high-weight molecular compounds to promote DOC persistence (Ding et al. 2020). Microorganisms metabolically consume DOC components and produce new microbial-derived compounds via the degradation of organic matter (Bowen et al. 2009). The consumption, transformation and production processes of microorganisms change DOC composition via preferential degradation of active molecules

with labile groups and cause the inert molecules to remain (Kalbitz et al. 2003). Both molecular assembly and microbial transformation are related to external factors (Li et al. 2018).

In our study, fertilization practices not only directly influence DOC composition, but also regulates the proportions of DOC groups via the mediation of soil physiochemical properties (Fig. 4). The pathways of fertilizer method that indirectly influenced the turnover of soil DOC components were mainly caused by adjustments in basic soil properties (e.g., soil pH, cation exchange capacity, and nutrient availability, Table S5), soil structure (e.g., aggregate stability, Table S6) and various forms of mineral ions (e.g., calcium and magnesium forms, Table S7). However, basic soil properties and mineral forms (including calcium and magnesium forms) were the primary mediators of fertilization effects on DOC turnover, while the mediation by soil aggregate was minimal (Fig. 5). This contrasts with some previous studies reporting a strong impact of soil aggregate on DOC (Chaplot and Cooper 2015; Cincotta et al. 2019; Xu et al. 2019). This discrepancy might be explained by the fact that the cohesive strengths of soil aggregate stability are from Van der Waals forces, hydrogen bonds or electrostatic attraction at atomic or molecular scales (Abiven et al. 2009). Relevant to this study are the cohesive forces of the calcium bridge, which is a much greater force than the cementing force of DOC molecules (Wuddivira and Camps 2007). Moreover, our results showed that our fertilization treatments had little influence on soil aggregate stability (Table S6), and the data also indirectly support that the strength of cohesion of aggregates in the gray desert soil likely comes from the internal calcium bonds (Jiang et al. 2017) and thus were not easily affected by external factors.

The variation in DOC composition was most strongly attributed to basic soil properties and forms of calcium and magnesium (Fig. 5), indicating that these two factors have a strong soil physiochemical imprint. Although basic soil properties and forms of calcium and magnesium explain DOC transformation well (Fig. 5), we found that the relationship between indices among basic soil properties and forms of calcium and magnesium and DOC groups differed (Fig. 3). Our results showed that the labile carbon-containing groups were positively associated with total nutrients and the stable carbon-containing groups were positively related to available nutrients. This finding supports the observation that nutrient availability is responsible for the utilization and transformation of stable groups by microorganisms (Nannipieri et al. 2017). Recent findings have demonstrated that nutrient availability fluctuates with changes in DOC characteristics in different soils (Creamer et al. 2016; Velescu et al. 2016). This highlights the regulated role of available nutrients in the soil carbon cycle, inhibiting the decomposition of organic matter in soils rich in available nutrients while promoting the decomposition of organic matter in soils lacking available nutrients (Dimassi et al. 2014). Microorganisms are the "creators" and "processors" of DOC. It has been demonstrated that the abundance of DOC molecules is significantly positively correlated with microbial diversity (Li et al. 2018). Moreover, microorganisms are also an important link connecting fertilization practice and turnover of DOC components (Hui et al. 2019). However, this study only focused on abiotic effects of soil physiochemical factors on the relationships between fertilization practice and DOC turnover. Therefore, the role that microorganisms play in DOC turnover in association with fertilization practices is an important direction for our future research.

5. Conclusions

Fertilization strategies affect DOC composition. Manure application promotes the turnover of aliphatic carbon to aromatic carbon, and chemical fertilizer application inhibits this turnover. Fertilization has both direct and indirect effects on DOC turnover, and basic soil physicochemical factors are the main mediators of the indirect effects. The availability of soil nutrients and magnesium minerals enhances DOC persistence via the enrichment of stable groups in DOC composition, and the availability of calcium minerals increases DOC availability via promotion of the accumulation of labile groups in DOC composition.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The authors declare that all relevant data supporting the findings of this study are available within the article and its supplementary information files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Yang Zhang, Shezhe E and Yanan Wang performed the field experiment and analyzed the field data regarding the chemical composition of dissolved organic carbon. Yang Zhang was a major contributor in writing the manuscript. Nan Zhang, Cuixai Wu and Xibai Zeng reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

FTIR spectrum (a) and ^{13}C -NMR spectrum (b) of DOC from five fertilization treatments (CT, no fertilization; NPK, chemical nitrogen, phosphorus and potassium fertilizers; M, pig and cattle manure; 1/2MNP, fertilization with 1/2 M and 1/2 NPK; MNPK, fertilization with total NPK and total M).

Figure 2

Carbon aliphatic and carbon aromaticity indices of DOC under influences of fertilization practices (CT, no fertilization; NPK, chemical nitrogen, phosphorus and potassium fertilizers; M, pig and cattle manure; 1/2MNPk, fertilization with 1/2 M and 1/2 NPK; MNPK, fertilization with total NPK and total M). * indicates a significant difference at $P < 0.05$. Error bars represent SEs.

Figure 3

Redundancy analysis of DOC composition (consisting of the N-alkyl, O-alkyl, alkyl, carbohydrate, phenolic, aryl and carbonyl C groups) on (a) basic soil properties (pH, soil acidity; σ , conductivity; CEC, cation exchange capacity; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, available nitrogen; AP, available phosphorus; AK, available potassium; and SOC, soil organic carbon), (b) calcium and magnesium forms (Free Ca, free calcium; Free Mg, free magnesium; Exchangeable Ca, exchangeable

calcium; Exchangeable Mg, exchangeable magnesium; Acid soluble Ca, acid-soluble calcium; Acid soluble Mg, acid-soluble magnesium; Residual Ca, residual calcium; Residual Mg, residual magnesium) and (c) aggregate stability indices (MWD, mean weight diameter; GMD, geometric mean diameter; FD, fractal dimension) under the influence of five fertilization practices (CT, no fertilization; NPK, chemical nitrogen, phosphorus and potassium fertilizers; M, pig and cattle manure; 1/2MNPK, fertilization with 1/2 M and 1/2 NPK; MNPK, fertilization with total NPK and total M).

Figure 4

The direct and indirect effects (the vectors to soil properties, aggregate stability, calcium forms and magnesium forms) of fertilization treatment on DOC composition (N-alkyl C, O-alkyl C, alkyl C, carbohydrate C, phenolic C, aryl C and carbonyl C). Black arrows represent indirect pathways (Path coefficient is 1). Blue arrows show negative effects and red arrows indicate positive effects; arrow thicknesses are proportional to the strengths of effects.

Figure 5

Variation partitioning for soil physicochemical factors (soil properties, aggregate stability, calcium forms and magnesium forms) of DOC transformation.

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