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Effects of Nitrogen Deposition on Soil Dissolved Organic Carbon and Nitrogen in Moso Bamboo Plantations Strongly Depend on Management Practices

Zhaofeng Lei [1,†], Huanfa Sun [1,†], Quan Li [1], Junbo Zhang [1] and Xinzhang Song [1,2,*]

1 State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou 311300, China; leizhaofeng777@163.com (Z.L.); sunhuanfa_zafu@163.com (H.S.); quanlizjafu@163.com (Q.L.); zhangjunbo12138@163.com (J.Z.)

2 Tianmu Mountain Forest Ecosystem Research Station, Hangzhou 311300, China

* Correspondence: songxinzhang@gmail.com; Tel.: +86-0571-6374-1816

† These authors contributed equally to this work and should be considered co-first authors.

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Abstract: Soil dissolved organic carbon (DOC) and nitrogen (DON) play significant roles in forest carbon, nitrogen and nutrient cycling. The objective of the present study was to estimate the effect of management practices and nitrogen (N) deposition on soil DOC and DON in Moso bamboo (*Phyllostachys edulis* (Carrière) J. Houz) plantations. This experiment, conducted for over 36 months, investigated the effects of four N addition levels (30, 60 and 90 kg N ha^{-1} $year^{-1}$, and the N-free control) and two management practices (conventional management (CM) and intensive management (IM)) on DOC and DON. The results showed that DOC and DON concentrations were the highest in summer. Both intensive management and N deposition independently decreased DOC and DON in spring ($p < 0.05$) but not in winter. However, when combined with IM, N deposition increased DOC and DON in spring and winter ($p < 0.05$). Our results demonstrated that N deposition significantly increased the loss of soil DOC and DON in Moso plantations, and this reduction was strongly affected by IM practices and varied seasonally. Therefore, management practices and seasonal variation should be considered when using ecological models to estimate the effects of N deposition on soil DOC and DON in plantation ecosystems.

Keywords: soil organic carbon; dissolved organic matter; nitrogen addition; *Phyllostachys edulis*

1. Introduction

Dissolved organic matter (DOM) is a mixture of organic molecules of different sizes and structures that can pass through a 0.45 μm sieve and dissolve in water and acidic and alkaline solutions [1,2]. Although DOM accounts for a small proportion of the soil organic matter pool, it plays an important role in microbial growth and metabolism, which regulate soil nutrient loss and affect the decomposition and transformation of soil organic matter [3,4]. As the two important components of DOM, dissolved organic carbon (DOC) and nitrogen (DON) are the two common empirical indices that reflect the quantitative characteristics of DOM [5]. DOC affects the regulation of cation leaching, mineral weathering, soil microbial activity, and anion adsorption and desorption, as well as other soil chemical, physical and biological processes [6]. DOC is a very important and active factor associated with terrestrial and aquatic ecosystems in the geochemical carbon cycle [6,7]. DON plays a dual role in N cycling in terrestrial ecosystems: on the one hand, it can be directly absorbed by plants and therefore shorten the terrestrial nitrogen cycle; on the other hand, however, DON has high mobility and can cause pollution in aquatic ecosystems through surface runoff or leaching [8]. DOC and DON have been recognized as the key components of forest C, N and nutrient cycling [9] and are therefore receiving a great deal of attention by researchers.

With the intensification of human activities, global atmospheric N deposition has increased rapidly, and several global N models predict that the subtropical regions in south central China will be among the areas most severely affected by atmospheric N deposition in the coming decades [10]. N is one of the essential elements for plant growth; thus, N deposition is thought to be beneficial to the plant. However, a few studies have shown that only a small proportion of atmospheric N deposited in forest ecosystems is utilized by plants, and that the major proportion of N is fixed in the soil [11]. Previous studies have shown the effects of N deposition on soil DOC and DON in forests. Frey et al. [12] found that more than half of the ecosystem C storage in a hardwood stand was attributable to an accumulation of soil organic matter, indicating that the soil has been more responsive to N addition than tree growth. Furthermore, they thought N enrichment resulted in a shift in organic matter chemistry and microbial community, thereby impacting DOM. Findlay et al. [13] reported that N deposition induces a great loss of soil DOC. Based on N saturation experiments, Gundersen et al. [14] showed that N input can increase the stability of soil humus and promote bacterial growth, thereby leading to a decrease in soil DON. Tu et al. [15] observed that N deposition significantly reduces soil microbial biomass carbon (MBC) but increases DOC in Sinocalmus bamboo (*Neosinocalamus affinis* (Rendle) Keng f.) plantations. However, the effects of N deposition on soil DOC and DON in Moso bamboo (*Phyllostachys edulis* (Carrière) J. Houz) plantations remain unknown.

Because of their rapid growth rate and high annual regrowth rate after harvesting, Moso bamboo forests are the most important source of non-wood forest products in China; they cover an area of 4.43 million ha and represent 73.7% of the country's bamboo forest area and 84.0% of the global distribution of Moso bamboo [16,17]. Our long-term investigation based on the Eddy covariance method showed that the Moso bamboo plantation ecosystem has a high C uptake capacity and might play an important role in mitigating climate warming [17]. In recent decades, intensive management (IM) has been implemented in more than half of these bamboo plantations to increase economic benefits. IM includes the removal of understory weeds, application of fertilizers, and soil tilling [18]. Typically, conventional management (CM) requires the regular harvest of bamboo stems and shoots, without any of the IM practices mentioned [19]. Thus, IM can affect soil organic carbon (SOC) [20], soil microbial biomass [21], and enzyme activities [22], which may affect the soil DOM. Moso bamboo plantations are mainly distributed in subtropical China, which is suffering severe N deposition of 30–37 kg N ha^{-1} $year^{-1}$ [19]. Our previous study [21] found that IM and N deposition significantly increased soil MBC but decreased bacterial diversity, and the combination of management practices and N deposition had greater effects on soil microbial biomass and diversity than either practice system or N deposition independently, which may impact soil DOC and DON. However, the mechanism of DOM response to these complicated factors and subsequent C and N cycles in Moso bamboo plantations remains unknown.

We conducted a more than three-year-long field experiment in Moso bamboo plantations to test the following three hypotheses: (1) IM practices decrease soil DOC and DON; (2) N deposition decreases soil DOC and DON; and (3) the combined effects of N deposition and management practices on soil DOC and DON are stronger than the effects of each of these factors independently.

2. Materials and Methods

2.1. Study Site

The study site was in Qingshan Town, Lin'an City (30°14′ N, 119°42′ E), Zhejiang Province, China. It has a subtropical monsoon climate, with mean annual precipitation and mean annual temperature of 1420 mm and 15.6 °C, respectively. The area receives an average of approximately 1847 h of sunshine and 230 frost-free days annually.

The CM Moso bamboo forests were originally established in the late 1970s from native evergreen broadleaf forests in sites of similar topography (southwest slope of approximately 6°) and soil type. The soils are named yellow-red soil and classified as Ferrisols derived from granite [19]. IM practices were conducted in half of the CM Moso bamboo forests since 2001. In September of each year, the IM Moso bamboo forests were fertilized and then plowed to a depth of 0.3 m. The application of the nitrate of S-based compound fertilizer (N-P_2O_5-K_2O: 15%-6%-20%, 450 kg ha^{-1}) is equivalent to the annual addition of 67.5 kg N, 11.8 kg P, and 74.7 kg K per hectare [19].

2.2. Experimental Design and N Treatment

Twelve CM plots and 12 IM plots, each with an area of 20 m × 20 m, were established. Detailed information on the experimental design can be found in Song et al. [19]. The local background atmospheric N deposition rate is 30–37 kg N ha^{-1} $year^{-1}$, with an average NH_4^+:NO_3^- of 1.28 [23]. Therefore, NH_4NO_3 was used as the N source for the low-N (30 kg ha^{-1} $year^{-1}$) treatment (N30), medium-N (60 kg ha^{-1} $year^{-1}$) treatment (N60), and high-N (90 kg ha^{-1} $year^{-1}$) treatment (N90). Three replicate plots for each treatment and the control (N-free) were randomized for each management practice. From January 2013, appropriate quantities of NH_4NO_3 were dissolved in 10 L water and sprayed evenly onto the forest floor of the corresponding plot every month. Each control plot received 10 L of N-free water every month to balance the effects of the water added.

2.3. Soil Sampling and Measurement

The experimental plots were sampled in the spring (30 April), summer (25 July), and winter (29 December) of 2016. The monthly mean air temperatures and precipitation quantities during the study period are shown in Table 1. Five soil cores at a depth of 0–20 cm were randomly collected from each plot and mixed. The samples were kept in an incubator, brought to the laboratory, and then sieved through a 2 mm mesh to remove roots, plant residues and stones. Next, we weighed two samples of 20 g fresh soil each and named them A and B. A was for determining DOC and total dissolved nitrogen (TDN) concentrations, and B was for determining soil moisture content. A was extracted with distilled water (soil:water ratio, 2:1), shaken for 0.5 h (170 rpm) at 25 °C, centrifuged for 20 min at 3500 rpm, and then filtered through a membrane (0.45 μm, Millipore, Xingya Corporation, Shanghai, China) into a plastic bottle [24]. DOC and TDN concentrations were determined using a total organic carbon analyzer (TOC-V_{CPH}, Shimadzu Corporation, Kyoto, Japan), and NH_4^+-N and NO_3^--N concentrations were determined using the SmartChem 200 Discrete Analyzer. DON was calculated as the difference between TDN and NH_4^+-N and NO_3^--N concentrations, according to Li et al. [24]. The ppm units of DOC, TDN, NH_4^+-N, and NO_3^--N were converted to mg/kg by the following formula:

$$\mathrm{M} = \frac{P \times 50 \times 10}{20 \times (1 + S)} \quad (1)$$

where M is the combined value of DOC, TDN, NH_4^+-N and NO_3^--N in mg/kg; *P* is value of DOC, TDN, NH_4^+-N and NO_3^--N in ppm; 50 is the transformation factor from ppm to mg/kg; 10 is the dilution factor; 20 stands for 20 g of the soil sample; and *S* is the soil moisture content.

Table 1. Average monthly climatic data of the study site during the experimental periods in 2016.

Months	Total Monthly Precipitation (mm)	Average Monthly Air Temperature (°C)
April	352.5	11.49
July	244.8	19.34
December	67.2	3.46

2.4. Data and Statistical Analyses

One-way analysis of variance (ANOVA) and the least significant difference (LSD) method were used to determine the statistical significance of the differences in DOC and DON concentration among N addition treatments under the same management and season, between the two management practices under the same N addition treatment and season, and among three seasons under the same management and N addition treatment.

Two-way ANOVA was performed to evaluate the combined influence of N deposition and management practices in each season. All data were tested for homogeneity of variance and normality of distribution prior to conducting the ANOVA. The data satisfied the assumption of homogeneity of variance. These analyses were performed using SPSS 22.0 (SPSS Inc. Chicago, IL, USA) and SigmaPlot 12.5 for Windows.

3. Results

3.1. Soil DOC

In the CM plots, N deposition significantly reduced DOC concentration in April and July, but not in December (Figure 1). Moreover, in July, the DOC concentration under N60 treatment was significantly higher than that under N30 or N90. In the IM plots, the effect of N deposition on DOC concentration was similar to that in the CM plots in July (Figure 1). However, this effect was not significant in April and December. Nonetheless, a significant increase was observed under the N90 treatment.

The DOC concentrations in the CM plots were significantly higher than those in the IM plots in April and July, but not in December (Figure 1). Moreover, DOC concentration was significantly higher in July than in April and December under both management practices (Figure 1).

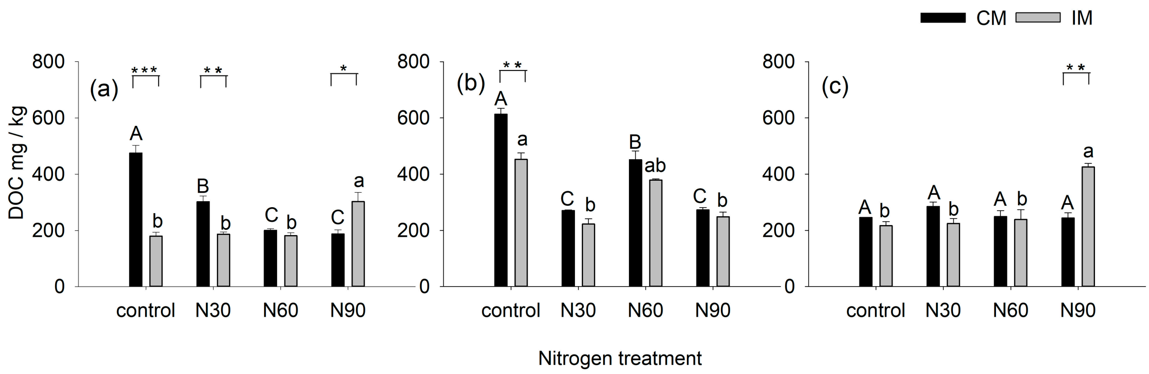

Figure 1. Dissolved organic carbon (DOC) in surface soil (0–20 cm) in different seasons ((**a**) April; (**b**) July; (**c**) December) under different management practices (CM: conventional management; IM: intensive management) and four nitrogen addition treatments (N30: 30 kg N ha^{-1} $year^{-1}$; N60: 60 kg N ha^{-1} $year^{-1}$; N90: 90 kg N ha^{-1} $year^{-1}$ and Control: N-free). Vertical bars indicate the standard error of three replicates. Different uppercase letters indicate significant differences among N addition rates under CM treatments ($p < 0.05$). Different lowercase letters indicate significant differences among N addition rates under IM treatments ($p < 0.05$). Asterisks indicate significant differences between CM and IM at the same N addition rate (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.2. Soil DON

In the CM plots, N deposition significantly decreased DON concentrations in April, but not in July and December, except for the N30 treatment in July ($p < 0.05$) (Figure 2). In the IM plots, only the N60 and N90 treatments significantly increased DON concentrations in both April and December, but not in July (Figure 2). The DON concentration was significantly higher in the CM plots than in the

IM plots in April, but not in July and December (Figure 2). Similar to DOC, DON concentration was significantly higher in July than in April and December, under both management practices (Figure 2).

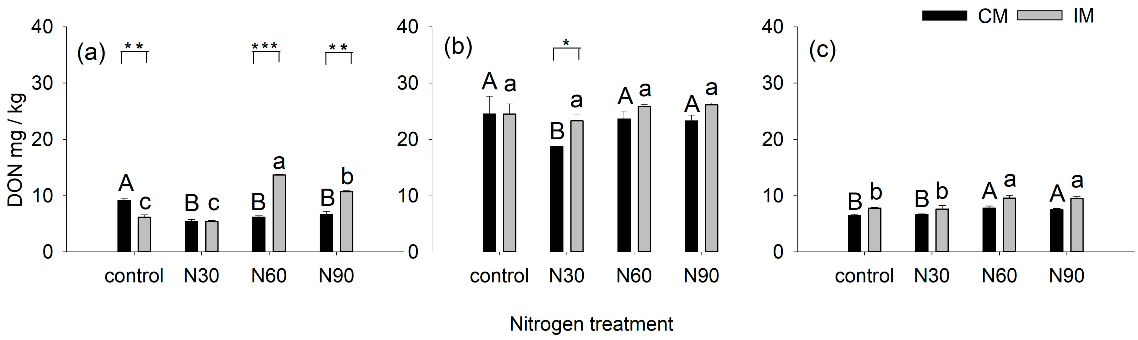

Figure 2. Dissolved organic nitrogen (DON) in surface soil (0–20 cm) in different seasons ((**a**) April; (**b**) July; (**c**) December) under different management practices (CM: conventional management; IM: intensive management) and four nitrogen addition treatments (N30: 30 kg N ha^{-1} $year^{-1}$; N60: 60 kg N ha^{-1} $year^{-1}$; N90: 90 kg N ha^{-1} $year^{-1}$ and Control: N-free). Vertical bars indicate the standard error of three replicates. Different uppercase letters indicate significant differences among N addition rates under CM treatments ($p < 0.05$). Different lowercase letters indicate significant differences among N addition rates under IM treatments ($p < 0.05$). Asterisks indicate significant differences between CM and IM at the same N addition rate (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.3. Combined Influence of N Deposition and Management on Soil DOC and DON

The two-way ANOVA showed that N deposition and management practices, independently and in combination, significantly affected DOC and DON in April ($p < 0.001$). In addition, the contribution of the interaction was greater than the independent effects of the two factors (Table 2). In July, N deposition and management practices, independently and in combination, significantly affected DOC ($p < 0.05$), whereas DON was significantly affected by the independent factors only ($p < 0.01$) (Table 2). Moreover, the contribution of separate factors was greater than of their interaction. In December, N deposition significantly affected the DOC only, whereas the management practices significantly affected DON only ($p < 0.05$), and their interaction significantly affected the DOC only (Table 2). The contribution of interaction was greater than that of the two factors separately on DOC only.

Table 2. Two-way ANOVA of the effects of N deposition and management practices on soil dissolved organic carbon (DOC) and nitrogen (DON) at 0–20 cm soil depths in Moso bamboo forests.

Months	Source of Variation/Factors	N Deposition			Management Practices			Interaction		
		F Value	*p* Value	Contribution (%) [a]	*F* Value	*p* Value	Contribution (%)	*F* Value	*p* Value	Contribution (%)
April	DOC	**18.98**	0.0000	22.00	**38.67**	0.0000	14.94	**49.07**	0.0000	56.88
	DON	**58.31**	0.0000	33.81	**74.61**	0.0000	14.42	**83.96**	0.0000	48.68
July	DOC	**66.26**	0.0000	82.92	**14.37**	0.0016	5.99	**3.53**	0.0390	4.42
	DON	**6.82**	0.0036	28.98	**32.99**	0.0000	46.74	0.38	0.7700	1.61
December	DOC	**6.23**	0.0052	26.46	0.36	0.5553	0.51	**11.87**	0.0002	50.38
	DON	2.30	0.1161	19.95	**7.54**	0.0143	21.80	1.39	0.2831	12.02

Significant contribution at $p < 0.05$ or $p < 0.01$ is shown in bold. [a] The contribution (%) is the percentage of overall variance explained by each factor.

4. Discussion

4.1. Effects of Management Practices on Soil DOC and DON

The present study showed that IM significantly decreased DOC and DON concentrations in spring (Figures 1 and 2), which partly supported our first hypothesis: IM practices decrease soil DOC and DON. Zhou et al. [25] showed that the total soil dissolved C at 0–20 cm soil depth in the IM Moso bamboo plantations was lower than that in the CM plots, which is consistent with the results of the present study. It is known that the concentrations of DOC and DON are mainly derived from ground litter, root exudates, soil humus, soil microbial biomass, and rainfall leaching [26]. Wu et al. [27] found that excessive consumption by microbial populations would decrease the DOC and DON in seasons with high temperature and high precipitation, which is consistent with our result. DOC and DON are important carriers of C and N loss in forest soil [28,29]. Furthermore, soil microbial consumption and leaching are the main output pathways of DOC and DON from forest ecosystems [30]. Yang et al. [31] proved that the growth of plant roots and soil microorganisms (represented by MBC) was enhanced by fertilization, increasing the amount of organic compounds (i.e., DOM) released by plant roots and soil microorganisms. Our previous study on this site showed that IM significantly increased soil MBC [21], indicating an increase in DOC consumption, which might greatly contribute to lower DOC concentrations in the IM plots than in the CM plots. Changed nutrient dynamics caused by management practices can also affect DOM concentrations between the native forests and plantations [27]. Long-term fertilization in the IM plots induced the loss of soil organic C and N and greatly decreased the chemical activity of the soil [20]. Soil acidification owing to long-term fertilization in the IM plots was more severe than that in the CM plots, which led to lower soil pH [19]. The decrease in soil pH might increase the adsorption capacity of Fe and/or Al oxides in soil [32], thereby reducing DOC and DON in the IM plots. Vance et al. [33] also reported a similar result. Generally, the DOM concentration is higher in forest topsoil than in cultivated soil, and plowing, weeding and fertilization in the IM plots alter the physical structure of soil, thus increasing the loss of DOC and DON through surface runoff and subsurface flow [34]. This, combined with high precipitation in spring (Figure 1), might lead to more export of DOC and DON from the IM plots and thereby decrease these concentrations more strongly in the IM than in the CM plots. The increased leaching effect on DOC in the rainy season was also observed by Neff and Asner [35]. Therefore, the high precipitation in spring (Table 1) might have washed a large amount of DOM and caused its loss by leaching, which might have contributed to the decline in DOC and DON between CM and IM in April and July.

In summer, DOC and DON concentrations were higher than those in spring and winter. However, IM largely reduced the concentration of DOC but not of DON in summer (Figures 1 and 2). Temperature can affect soil DOM concentration and turnover by controlling microbial biomass [36]. Jiang et al. [37] found that there was twice as much microbial biomass in summer than in spring in Moso bamboo plantations; thus, the high temperature in July (Table 1) might have contributed to the high DOC and DON. Furthermore, compared with spring, summer and winter had lower precipitation (Table 1), thereby inducing a smaller leaching loss of DOC and DON. At this point, the N input from fertilization might have contributed more to the slightly higher DON in the IM plots than that in the CM plots.

In winter, DOC and DON concentrations were low and did not show significant differences between IM and CM (Figures 1 and 2). A possible reason is that the low temperature in winter decreased the positive effects of temperature on DOC and DON. Moreover, the leaching loss of DON induced by plowing in the IM plots might have declined due to low precipitation (Table 1) and might have even been offset by N input from fertilization, which can contribute to slightly higher DON in the IM plots than that in the CM plots.

4.2. Effects of N Deposition on Soil DOC and DON

In the present study, N addition significantly decreased DOC and DON in the CM plots in spring (Figures 1 and 2), which partly supports our second hypothesis: N deposition decreases soil DOC and DON. N saturation experiments showed that exogenous N input can increase the leaching loss of DON [14] and induce a decrease in soil DON. Previous studies have reported that long-term N deposition reduces soil MBC [22,38]. A high degree of N deposition could affect the composition of the microbial community and inhibit the C of microbial degradation, thereby decelerating the decomposition of litter [39,40]. The effect mentioned above contributes to a decline in soil DOC and DON. Notably, N deposition can intensify soil acidification and decrease soil pH [21], which can potentially change the acidity of soil solutions [41–44]. This, in turn, may lead to the decline in DOC and DON in the CM plots.

In summer, N deposition significantly decreased the DOC concentration but did not significantly affect DON, except under the N30 treatment (Figures 1 and 2). In contrast to DOC, DON increased substantially from April to July, which might be attributed to the stronger adsorption of soil to DON than DOC at high temperatures [45]. Moreover, the high-temperature effect might be offset by the negative effect of N deposition on DON. DOC and DON concentrations in winter were not affected by N deposition (Figures 1 and 2). Probably, the low soil microbial activity due to low temperature and precipitation in winter alleviated the effect of N deposition.

4.3. Combined Influence of N Deposition and Management Practices on Soil DOC and DON

When combined with IM, N deposition significantly increased DOC and DON concentrations in spring and winter. This effect was opposite to that of the independent effect of N deposition in the CM plots. This result indicated that intensive management practices may change the direction of the effects of N deposition on DOC and DON (Figures 1 and 2), which did not support our third hypothesis: the combined effects of N deposition and management practices on soil DOC and DON are stronger than the effects of each of these factors independently. Our previous study at this site found that high N deposition decreased the decomposition of leaf litter and fine roots in IM plots [19,23], which could lead to more litter and fine root accumulation on the soil surface and subsurface. This accumulation may alleviate the leaching of DOC through surface runoff and subsurface flow, which partially explains the current finding that high N addition increased DOC in the IM plots. Nevertheless, in summer, the combined effects of N deposition and IM on DOC and DON were similar to the independent effect of N deposition in the CM plots (Figures 1 and 2), which indicated that the effect of interaction of N deposition and management practices on DOC and DON can vary with season.

The two-way ANOVA demonstrated that in spring, the combined influence of N deposition and management practices had greater effects on soil DOC and DON than the effects of each of these factors independently, which supported our third hypothesis (Table 2). Our previous study [21] at this site demonstrated that differences in microbial community structure were primarily due to a combination of N deposition and management practices (57.73%), with management practices alone accounting for 36.26% of the variation and N addition accounting for 21.47%, indicating that the combination of two factors has a stronger impact on DOM than each factor singly, which also supported our present result. In summer, the positive effects of high temperature partially offset the negative effects of N deposition and IM alone on DOC and DON, thus contributing to the low combination of these two factors (Table 2). Our previous study at this site elucidated that N deposition significantly decreased the diversity of soil microorganisms in both CM and IM plots [21], which indicates that the soil DOC and DON may be strongly correlated with microbial diversity in Moso bamboo plantations. Our results suggest, however, that N deposition has a higher contribution than management practices to soil DOC and DON (Table 2), except for DON in summer. Both DOC and DON concentrations showed the same tendency in the control treatment or the combined effects of N deposition and management practices: DOC and DON increased from spring to summer, then decreased in winter. It appears to be the combination of high precipitation and temperature that increased soil adsorption to DOC, especially for DON [45].

5. Conclusions

The present study showed that DOC and DON concentrations were higher in summer than in spring and winter in Moso bamboo plantations. Both IM practices and N deposition independently decreased DOC and DON in spring, but not in winter. When combined with IM, N deposition increased DOC and DON in spring and winter. The effects of N deposition on soil DOC and DON strongly depended on management practices and season, suggesting that management practices and seasonal variation should be taken into account when applying ecological models to estimate the effects of N deposition on DOC and DON in terrestrial ecosystems, also indicating that anthropogenic management practices such as plowing and weeding would partially offset the negative effects of N deposition on DOM in the Moso bamboo plantation ecosystem. The results of the present study provide a new perspective for improving our understanding of the comprehensive effects of N deposition and management practices on C and N cycling in plantations. Our findings also offer a beneficial reference on how to manage plantations under the current background of increasing levels of atmospheric N deposition.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/8/11/452/s1, Figure S1: Ammonium nitrogen (NH_4^+-N), Nitrate nitrogen (NO_3^--N) and Total dissolved nitrogen (TDN) in surface soil (0–20 cm) in different seasons ((a), (d), (g): April; (b), (e), (h): July; (c), (f), (i): December) under different management practices (CM: conventional management; IM: intensive management) and four nitrogen addition treatments (N30: 30 kg N ha^{-1} $year^{-1}$; N60: 60 kg N ha^{-1} $year^{-1}$; N90: 90 kg N ha^{-1} $year^{-1}$ and Control: N-free). Vertical bars indicate the standard error of three replicates. Different uppercase letters indicate significant differences among N addition rates under CM treatments ($p < 0.05$). Different lowercase letters indicate significant differences among N addition rates under IM treatments ($p < 0.05$). Asterisks indicate significant differences between CM and IM at the same N addition rate (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$), Table S1: The initial stand and soil characteristics of the study sites in the Moso bamboo forest (mean ± SD, $n = 4$).

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Author Contributions: Z.L. and H.S. analyzed the data and drafted the manuscript, and participated in collecting the experiment data. Q.L. and J.Z. were involved in data sampling. X.S. designed this experiment and drafted the manuscript. All authors discussed the results and revised the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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