Nitrogen addition stimulates soil aggregation and enhances carbon storage in terrestrial ecosystems of China: A meta-analysis

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Abstract
China is experiencing a high level of atmospheric nitrogen (N) deposition, which greatly affects the soil carbon (C) dynamics in terrestrial ecosystems. Soil aggregation contributes to the stability of soil structure and to soil C sequestration. Although many studies have reported the effects of N enrichment on bulk soil C dynamics, the underlying mechanisms explaining how soil aggregates respond to N enrichment remain unclear. Here, we used a meta-analysis of data from 76N manipulation experiments in terrestrial ecosystems in China to assess the effects of N enrichment on soil aggregation and its sequestration of C. On average, N enrichment significantly increased the mean weight diameter of soil aggregates by 10%. The proportion of macroaggregates and silt-clay fraction were significantly increased (6%) and decreased (9%) by N enrichment, respectively. A greater response of macroaggregate C (+15%) than of bulk soil C (+5%) to N enrichment was detected across all ecosystems. However, N enrichment had minor effects on microaggregate C and silt-clay C. The magnitude of N enrichment effect on soil aggregation varied with ecosystem type and fertilization regime. Additionally, soil pH declined consistently and was correlated with soil aggregate C. Overall, our meta-analysis suggests that N enrichment promotes particulate organic C accumulation via increasing macroaggregate C and acidifying soils. In contrast, increases in soil aggregation could inhibit microbially mediated breakdown of soil organic matter, causing minimal change in mineral-associated organic C. Our findings highlight that atmospheric N deposition may enhance the formation of soil aggregates and their sequestration of C in terrestrial ecosystems in China.

KEYWORDS
carbon cycle, global change, meta-analysis, nitrogen deposition, soil aggregation, soil carbon sequestration
1 | INTRODUCTION

Atmospheric nitrogen (N) deposition caused by agricultural activities and fossil fuel combustion, which together are creating reactive N at an accelerating rate, has been dramatically increasing over the past decades (Galloway et al., 2008; Penuelas et al., 2013). Chronic N deposition can help mitigate the negative effects of anthropogenic carbon dioxide (CO\text{2}) emission by increasing carbon (C) sequestration in terrestrial ecosystems (Bala et al., 2013; Frey et al., 2014; Magnani et al., 2007; Zak et al., 2017). Terrestrial C pools are important components of the global C cycle, with soil being a major terrestrial C pool (Post et al., 1982; Schimel, 1995). Soil C pools were estimated to accumulate 5%-15% of the annual global C emissions (Lal, 2004). Minor changes in the soil C pools greatly affect the global C balance and lead to global climate change (Jandl et al., 2007; Stockmann et al., 2013). A better understanding of the effects of atmospheric N deposition on the dynamics of soil C pools should therefore be a research priority.

N-induced soil C sequestration has been widely investigated. Many experiments (e.g., Argiroff et al., 2019; Frey et al., 2014) and meta-analyses (e.g., Janssens et al., 2010; Liu & Greaver, 2010; Lu et al., 2011) have documented positive, neutral, and negative effects of N enrichment on soil C accumulation. However, the underlying mechanisms of how N regulates soil C budgets remain uncertain, and this uncertainty has generated extensive debate about aboveground and belowground C allocation and the interrelationships between them under N enrichment (Carvalho et al., 2017; Liu & Greaver, 2010; Song et al., 2019; Sullivan et al., 2007). Many studies have confirmed that adequate N addition can stimulate plant growth and increase aboveground C storage, because most terrestrial ecosystems in temperate regions are N limited (Fernandez-Martinez et al., 2014; Schulte-Uebbing & de Vries, 2018; Yan et al., 2019). In contrast, other studies found that the belowground C stock was not always linearly correlated with aboveground C process under N-addition treatments, despite about 50% C fixed annually by plants is inputted into soil. For instance, a meta-analysis of 512 pairs of observations showed that although N addition substantially increased the aboveground C stock (more than 35%) and organic matter input to soils, soil C storage was not significantly enhanced by N addition in forests or grasslands (Lu et al., 2011), a finding that was supported by other researchers (Song et al., 2019; Sullivan et al., 2007). A growing body of evidence suggests that complex soil carbon-related processes caused by excessive N addition lead to the uncoupling of aboveground-belowground C dynamics (Deng et al., 2018; Liu & Greaver, 2010). It follows that studies on the effects of N deposition on soil C sequestration should probably focus more on belowground rather than on aboveground C process.

Soil microbial degradation is largely responsible for belowground C decomposition and can be greatly affected by N deposition (Bonner et al., 2019; Zhou et al., 2017). N-induced soil acidification, high osmotic pressure, and Al\text{3+} toxicity limit microbial growth and extracellular enzyme activities (Hogberg et al., 2006; Tian & Niu, 2015; Treseder, 2008), leading to decreases in microbial biomass C, microbial diversity, and the activities of oxidases (Jian et al., 2016; Wang et al., 2018). These inhibitory effects would be expected to suppress soil organic C (SOC) decomposition. In contrast, increased availability of N and/or C caused by N addition could increase microbial biomass, especially in N-limited ecosystems. For example, by increasing plant growth and the concentration of N in plant tissue (LeBauer & Treseder, 2008; Liu et al., 2013), N addition could increase the quantity of litterfall and the N content of the litterfall, which could increase microbial C utilization, resulting in increased microbial products and increased formation of stable soil organic matter at the initial stage of decomposition (Cotrufo et al., 2013). Hydrolytic enzymes are generally responsible for the microbial degradation of simple compounds and for N acquisition. In previous studies, N fertilization at appropriate rates increased hydrolytic enzyme activities and microbial growth (Jian et al., 2016; Zhou et al., 2017). Given the different responses of microbial communities to N deposition, however, the effects of N addition on soil C dynamics remain unclear and require further investigation in different ecosystems.

Soil aggregates are the main components of soil structure and are classified by diameter into macroaggregates (>250 \text{μm}), microaggregates (53–250 \text{μm}), and the silt-clay fraction (<53 \text{μm}). Macroaggregates contain of plant residues and form the particulate organic C (POC) pool, while the mineral-associated organic C (MOC) pool is linked to silt-clay fraction (Ahn et al., 2009; Blanco-Canqui & Lal, 2004; Six et al., 2000). Because soil aggregates physically protect the organic carbon against microbial degradation (Balesdent et al., 2000), the stability of soil aggregates substantially influences soil C sequestration (Smith et al., 2014). The mean weight diameter (MWD) is an index for which the different size classes are weighted by their mean fraction size. Thus, the larger MWD usually means more stable particles in the analyzed sample. Depending on wet or dry sieving, the resulting MWD better reflects the stability of soil structure (Tripathi et al., 2008). Some studies have shown that N deposition can help stabilize soil structure and reduce POC decomposition by increasing the formation of macroaggregates and MWD (Chang et al., 2019; Zak et al., 2017; Zhong et al., 2017), but other studies have found that N deposition increases POC decomposition (Chen et al., 2019; Luo et al., 2020; Sun et al., 2018). Similarly, the wide-ranging effects of N enrichment on MOC (i.e., positive, negative, and neutral) preclude broad consensus (Cusack et al., 2011; Riggs et al., 2015).

The various responses of POC and MOC to N addition may explain the uncoupled aboveground-belowground C processes (Averill & Waring, 2018; Ye et al., 2018). Abiotic factors such as phosphorus (P) and N availability (Luo et al., 2020), precipitation (Chen et al., 2019), and fine root properties (Leifheit et al., 2014) are thought to affect the stability of soil aggregates and therefore C storage. Multiple soil microbial processes have been proposed to explain the changes in the POC and MOC pools in response to N addition (Averill & Waring, 2018; Bonner et al., 2019; Ye et al., 2018). The reported effects of N addition on soil aggregates have been inconsistent, probably because of differences in N treatment...
conditions and ecosystem types. Although soil aggregates have been extensively investigated, large knowledge gaps remain regarding the mechanisms by which N enrichment affects C sequestration in soil aggregates.

China is experiencing intense N-emitted pollution and has become the largest emitter of reactive N worldwide (Liu et al., 2011, 2013). The average annual bulk N deposition increased from 13.2 in 1980 to 21.1 kg N ha\(^{-1}\) in 2010 (Liu et al., 2013), and is continuing to increase. Because the country contains multiple types of ecosystems and a wide range of climatic conditions, the effects of atmospheric N deposition on terrestrial ecosystems in China are varied. To date, several meta-analyses have considered the effects of N enrichment on belowground C dynamic or SOC accumulation in China (e.g., Chen et al., 2015; Deng et al., 2018). However, little research has been conducted on the effects of N enrichment on soil aggregates. Considering the importance of soil aggregates to the soil C pool (Blanco-Canqui & Lal, 2004), further study is necessary to determine how N deposition influences soil aggregates and C sequestration within soil aggregates. Here, we conducted a meta-analysis to quantify the responses of soil aggregates and C sequestration within aggregates to N enrichment in terrestrial ecosystems in China. We attempted to answer two questions: (1) How is the formation of soil aggregates and C sequestration within aggregates affected by N deposition? (2) What are the underlying mechanisms that explain the effects of N deposition on C sequestration within soil aggregates?

2 | MATERIALS AND METHODS

2.1 | Data selection

We constructed our dataset by searching peer-reviewed publications from Web of Science (WoS, 1 January 2000 to 30 April 2020) that reported manipulative N-addition experiments in terrestrial ecosystem in China. On-line searching spanned May-July 2020 at Nanjing University. Databases used for the search included (1) WoS Core Collection, (2) BIOSIS Citation Index, (3) Chinese Science Citation Database, (4) Derwent Innovations Index, (5) KCI-Korean Journal Database, (6) MEDLINE, (7) Russian Science Citation Index, and (8) SciELO Citation Index. Articles reporting responses of soil aggregation and aggregate C to N addition were selected to explain the effect of N enrichment on soil C sequestration. According to insight from previous meta-analysis (e.g., Chen et al., 2015; Deng et al., 2018) and a priori test, the keyword combinations used for title searching in WoS were “(nitrogen) AND (deposition or addition or enrichment or fertilizer or elevated) AND (soil carbon or soil aggregates or soil structure or soil sequestration or soil storage).” Considering that soil microbes are strongly linked with decomposition of soil organic matter, we also collected the articles that reported the responses of soil microbial communities to better explain the potential mechanisms of N-induced soil aggregation. The keyword combinations used for title searching in WoS were “(nitrogen) AND (deposition or addition or enrichment or fertilizer or elevated) AND (soil microbes or microbial communities or microbial biomass or extracellular enzyme).”

To avoid bias, we selected articles that met the following criteria: (1) the data were directly obtained from N-addition experiments in China, that is, reviews, modeling studies, and greenhouse experiments were excluded; (2) control and N-treatment plots were established at the same experimental site with the same climate, soil, and vegetation types, and at least one of the critical variables (i.e., soil aggregation, soil aggregate C, soil microbial communities, or extracellular enzymes) was measured; (3) the means, standard deviation (SD), and replication number of selected variables could be directly obtained or calculated from the article or could be extracted from digitized graphs with Getdata Graph Digitizer (version 2.26, Moscow, Russia). If the study used standard error (SE) rather than SD, SD was calculated as SE \(\sqrt{n}\). If the study did not report SD or SE, the missing SD was calculated by multiplying the reported mean by the average coefficient of variance of the complete dataset (Weir et al., 2018); (4) When one study involved more than one observation from different sampling times, the latest measurements were adopted. Data from different N-addition rates were considered as multiple observations in the same experiment. Lu and Guo screened articles (double checked each other) and found 73 published articles that met our criteria for this meta-analysis. Three known unpublished studies were also included (one from grassland and two from forests). We draw a PRISMA flow diagram (Figure S5) to show the procedure of the article selection. A total of 76 studies with 1462 pairs of observations were collected in our datasets across major terrestrial ecosystems in China (Figure 1, see Supplementary Dataset S1).

2.2 | Data compilation

We used a common meta-analysis method as described in detail by Hedges et al. (1999) to evaluate the responses of selected variables (Figure 1) to N deposition. The effect size was calculated by the natural log-transformed response ratio (RR):

\[
\ln RR = \ln \left( \frac{\bar{X}_N}{\bar{X}_C} \right) = \ln (\bar{X}_N) - \ln (\bar{X}_C),
\]

where \(\bar{X}_N\) is the mean values of the N treatment group, and \(\bar{X}_C\) is the mean values of the control group. The variance (\(v\)) of each lnRR was calculated as follows:

\[
v = \frac{s_t^2}{n_t \bar{X}_t^2} + \frac{s_c^2}{n_c \bar{X}_c^2},
\]

where \(s_t\) and \(s_c\) are the standard deviations of the N-addition treatment and control group, respectively, and \(n_t\) and \(n_c\) are the sample sizes of the N-addition treatment and control group, respectively.

The data extracted from selected studies comprised five groups: (1) soil aggregation (i.e., the proportion of macroaggregates...
[>250 μm], microaggregates [53–250 μm], silt-clay fraction [<53 μm], and the MWD), (2) soil C (i.e., bulk soil C, macroaggregate C, microaggregate C, and silt-clay C), (3) other soil chemical properties (i.e., soil pH, total nitrogen, ammonia nitrogen, and nitrate nitrogen), (4) soil microbial communities (i.e., microbial biomass C, microbial biomass N, total phospholipid fatty acid, biomass of fungi, and biomass of bacteria), and (5) the activities of extracellular enzymes (i.e., cellulose, β-1,4-glucosidase, polyphenol oxidase, and peroxidase). We addressed the question “How is the formation of soil aggregates and C sequestration within aggregates affected by N deposition?” via analyzing the response of “soil aggregation” and “soil aggregate C” to N enrichment. Then, the “soil properties,” “soil microbial communities,” and “extracellular enzymes” were used to explore the potential mechanisms of N-induced soil C dynamics, and to address the question “What are the underlying mechanisms that explain the effects of N deposition on C sequestration within soil aggregates?”.

For each observation, we included additional information on characteristics of the sampled plot (latitude and longitude, mean annual temperature [MAT], and mean annual precipitation [MAP]), ecosystem types, and fertilization regimes (N-addition rate, N fertilizer form, and experimental duration). Previous meta-analysis studies suggest that the response of C-related processes to N enrichment can vary with ecosystem types and fertilization regimes (Chen et al., 2015; Deng et al., 2018; Lu et al., 2011). Thus, all of observations were subdivided into four categories based on ecosystem type (subtropical forest, temperate forest, grassland, and cropland), N-addition level (high, >100; medium, 50–100; low, <50 kg N ha⁻¹ year⁻¹), N fertilizer form (organic, i.e., urea; inorganic, i.e., NH₄NO₃, NaNO₃, NH₄Cl), and experimental duration (<5, 5–10, and >10 years). We chose these thresholds for the level breakpoints based on the previous meta-analysis studies (e.g., Song et al., 2019; Wang et al., 2018) and general level breakpoints of manipulative N-addition experiments in our dataset.

2.3 Statistical analyses

A two-step method described by Song et al. (2020) was used to handle within-paper non-independence in observations, that is, a
weighted mean value was calculated from multiple observed effect sizes in each study, which was then used to calculate the overall effect size using a standard random-effect method. Moulin and Amaral (2020) suggest that studies performed within a research group are more similar to each other than studies performed from different research groups. To deal with this kind of non-independency, we used the R package “bibliometrix” version 3.0 (Aria & Cuccurullo, 2017) to construct author networks, and then manually assigned papers to their respective clusters (76 studies in 44 clusters, Figure S8). The author cluster information was implemented in the meta-analysis model. The weighted effect size \( \bar{RR} \) was calculated as follows:

\[
\ln \bar{RR} = \frac{\sum_{i=1}^{m} w_i \ln (RR_i)}{\sum_{i=1}^{m} w_i},
\]

where \( m \) is the number of comparisons in the group (e.g., ecosystem types), and \( w_i \) is the weighting factor of the \( i \)th experiment in the group. \( w_i \) was calculated as follows:

\[
w_i = \frac{1}{v_i},
\]

\[
v_i = v + T_i^2,
\]

where \( v \) is the variance of study \( i \) in the group, \( v \) is the within-study variance of study, and \( T_i^2 \) is the between-studies variance. The standard error of \( \bar{RR} \) and 95% confidence interval (CI) were calculated as follows:

\[
s(\ln \bar{RR}) = \sqrt{\frac{1}{\sum_{i=1}^{m} w_i}},
\]

\[
95\% \ CI = \ln \bar{RR} \pm 1.96s(\ln \bar{RR}).
\]

The responses of selected variables to N deposition were considered significant at \( p < 0.05 \) if the 95% CI of \( \bar{RR} \) did not overlap 0. We also used the percentage change transformed from \( \bar{RR} \) to better explain the response of selected variables to N addition:

\[
\text{Effect size (%) = } (e^{\ln \bar{RR}} - 1) \times 100\%.
\]

We used a random-effects meta-analysis model to calculate weighted effect sizes \( \bar{RR} \) and to implement subgroups (ecosystem type, N-addition level, N form, or experimental duration) using the “rma.mv” function in “metafor” package version 2.4 (Viechtbauer, 2010) in R software (version 4.2.0, R Development Core Team, 2019). To assess the significance of each subgroups, total heterogeneity \( (Q_w) \) in each category was classified into within-group \( (Q_w) \) and between-group \( (Q_g) \) at two levels. It indicated a significant difference among different levels in each category if there was a significant difference in \( Q_w \). A single linear regression was used to estimate the relationship between soil aggregates, soil pH, and MBC. In addition, to determine whether ambient conditions (MAT and MAP) affect the formation of soil aggregates and their C concentration, we grouped observations according to MAT (high, >20; medium 10–20; low, <10°C) and MAP (high, >1600; medium, 800–1600; low, <800 mm) of the sampled plots. A general linear model was used to analyze the effects of ambient conditions on soil aggregation and soil aggregate C. A logit transformation was used to handle with the proportional data before running the meta-analysis.

We assessed the publication bias by funnel plots using “funnel” function (Figure S6), and examined the robustness of our calculated effect sizes by performing “leave-one-out” tests using “leave1out” function (Figure S7). We found that publication bias in our meta-analyses was mostly nonsignificant, and our calculated effect sizes were mostly robust (more details in Figures S6 and S7). Significance was set at \( p < 0.05 \).

3 | RESULTS

3.1 | Effects of N enrichment on soil aggregation

Across all ecosystem types and as indicated by effect sizes, N enrichment significantly increased the MWD in comparison to the control. The proportion of macroaggregates, microaggregates, and silt-clay fraction was significantly increased, neutral, and significantly decreased by N addition, respectively (Figure 2). When subdivided into different ecosystem types, MWD was significantly increased by N addition in temperate forests and grasslands while it was not significant in other ecosystems (Figure 3d). In addition, the proportion of silt-clay fraction was only significantly
decreased by N addition in subtropical and temperate forests (Figure 3c).

To different fertilization regimes, MWD and microaggregates were significantly affected by N fertilizer form (Table S1, p < 0.05). The proportion of microaggregates was significantly increased by inorganic N fertilizer, but was not significantly affected by organic N fertilizer (Figure 3b). Both organic and inorganic N fertilizer significantly enhanced the MWD, but MWD in organic fertilizer was significantly higher than that in inorganic fertilizer (Figure 3d). In addition, MWD was significantly affected by experimental duration (Table S1, p < 0.05). Short-term and medium-term N treatment significantly increased the MWD, but long-term N treatment had a minor stimulation of MWD.

3.2 | Effects of N enrichment on the C concentration in soil aggregates

As indicated by effect sizes, N enrichment significantly increased bulk soil C and macroaggregate C, but did not significantly affect microaggregate C and silt-clay C (Figure 2). The C concentration in aggregates was affected by ecosystem type and N-treatment conditions (Table S1). To different ecosystems, N addition significantly increased macroaggregate C in subtropical forests, temperate forests, and grasslands, but did not significantly affect it in croplands (Figure 4a). The microaggregate C and silt-clay C were only significantly increased in temperate forests by N addition (Figure 4b,c).

To different fertilization regimes, the form of N fertilizer significantly affected the microaggregate C and silt-clay C. They both increased by organic N fertilizer, but did not significantly affect by inorganic N fertilizer (Figure 4b,c). Experimental duration significantly affected the macroaggregate C and bulk soil C. Macroaggregate C was significantly increased by short-term and long-term N treatments, but it was not significantly affected by medium-term N treatments (Figure 4a). The opposite trend was generally true for bulk soil C (Figure 4d).

3.3 | Effects of N enrichment on soil and microbial properties

As indicated by effect sizes, N enrichment significantly decreased soil pH, whereas it significantly increased soil total N, nitrate N, and ammonia N (Table 1). N enrichment significantly decreased microbial
biomass C and increased microbial biomass N. N enrichment significantly decreased fungal biomass but did not significantly affect the biomass of total PLAF and bacteria. The responses of extracellular enzyme activities to N addition were inconsistent. N enrichment had a minor effect on the activities of hydrolytic enzymes (i.e., cellulase, β-1,4-glucosidase), but significantly decreased the activity of oxidase enzyme (i.e., peroxidase).

3.4 Relationships between soil aggregates and abiotic/biotic factors

Regression analysis showed that the response ratios of macroaggregate C ($R^2 = 0.35, p < 0.01$) and microaggregate C ($R^2 = 0.23, p < 0.01$) decreased with the response ratio of soil pH (Figure 5a,e); the response ratio of silt-clay C tended to increase with the response ratio of soil pH, but the correlation was not statistically significant. Unexpectedly, there was no significant correlation between the response ratio of soil aggregation and soil pH except for macroaggregates ($R^2 = 0.16, p < 0.05$). Both the C concentration ($R^2 = 0.16, p < 0.05$) and the proportion of macroaggregates ($R^2 = 0.27, p < 0.05$) were negatively related with the response ratio of MBC (Figure 5c,d).

### TABLE 1 Effects of N enrichment on soil properties, microbial communities, and extracellular enzyme activities

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect size (%)</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil properties</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil pH</td>
<td>-4.9 [-7.4 to -2.4]</td>
<td>105 (29)</td>
</tr>
<tr>
<td>Total N</td>
<td>6.7 [1.4 to 12.2]</td>
<td>80 (22)</td>
</tr>
<tr>
<td>Nitrate N</td>
<td>96.5 [40.2 to 175.4]</td>
<td>61 (14)</td>
</tr>
<tr>
<td>Ammonia N</td>
<td>44.8 [17.4 to 78.7]</td>
<td>61 (14)</td>
</tr>
<tr>
<td>Microbial communities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial biomass C</td>
<td>-12.1 [-22.6 to -0.2]</td>
<td>98 (27)</td>
</tr>
<tr>
<td>Microbial biomass N</td>
<td>22.3 [4.1 to 43.8]</td>
<td>62 (15)</td>
</tr>
<tr>
<td>Total PLFA</td>
<td>-4.5 [-21.1 to 15.5]</td>
<td>58 (17)</td>
</tr>
<tr>
<td>Fungi</td>
<td>-13.6 [-24.1 to -1.8]</td>
<td>64 (17)</td>
</tr>
<tr>
<td>Bacteria</td>
<td>2.3 [-18.5 to 28.5]</td>
<td>53 (15)</td>
</tr>
<tr>
<td>Extracellular enzyme activities</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulase</td>
<td>2.2 [-16.2 to 24.7]</td>
<td>46 (10)</td>
</tr>
<tr>
<td>β-1,4-glucosidase</td>
<td>2.2 [-10.3 to 16.5]</td>
<td>58 (15)</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>-12.1 [-20.9 to -2.2]</td>
<td>34 (7)</td>
</tr>
<tr>
<td>Polyphenol oxidase</td>
<td>-6.4 [-17.6 to 6.3]</td>
<td>47 (10)</td>
</tr>
</tbody>
</table>

Note: Numbers in the square brackets are 95% confidence intervals (CI). Sample size is shown as number of observations (number of studies).
MAT and MAP significantly affected macroaggregate C and silt-clay C but not significantly affected soil aggregation (Table S2).

4 | DISCUSSION

4.1 | N enrichment promoted the soil aggregation

Our meta-analysis revealed that exogenous N input increased the MWD (Figure 2), suggesting a positive effect of N addition on the soil structure stability (Tripathi et al., 2008). Similar results were reported in many field experiments (Chang et al., 2019; Liu et al., 2015; Zhong et al., 2017), but different results were also found in other studies (Chen et al., 2019; Sun et al., 2018; Wu et al., 2018). We found the effect of N addition on soil aggregation is complex. Based on the hierarchical model of soil aggregation (Six et al., 2000; Tisdall & Oades, 1982), small diameter aggregates can join to form large diameter aggregates, and large diameter aggregates can separate into small diameter aggregates. The different effects of N enrichment on the proportion of macroaggregates (+6%), microaggregates (neutral), and the silt-clay fraction (~9%) indicate that N enrichment facilitates the formation of large diameter aggregates.

Soil aggregation depends on organic binding agents, which include temporary agents (roots and fungal hyphae) that mainly stabilize macroaggregates, transient agents (polysaccharides, produced by soil microorganisms and roots) that commonly bind the silt-clay fraction, and persistent agents (highly humified organic matter, polyvalent metal cations, and adsorbed complexes) that are associated with microaggregates (Blanco-Canqui & Lal, 2004; Tisdall & Oades, 1982). A possible explanation of why N addition increased the macroaggregation but decreased the proportion of the silt-clay fraction is that N addition influences the production of organic binding agents. N enrichment increases litter and root biomass (Li et al., 2015; Lu et al., 2011), which facilitates aggregate stability (Six et al., 1999). Litter residue and root exudates can bind with fungal hyphae and soil particles to form macroaggregates (Tisdall & Oades, 1982). However, N enrichment had a negative effect on fungi (Table 1). Leifheit et al. (2014) reported that arbuscular mycorrhizal fungi (AMF) increased soil aggregation, and N inhibition of fungi, especially of AMF, might therefore reduce the formation of macroaggregates (Jia et al., 2020; Zhou et al., 2017). The transient and temporary binding agents contribute to the combining of the silt-clay fraction (Blanco-Canqui & Lal, 2004). Inhibition of microbial growth by N enrichment documented in our meta-analysis (Table 1) and in a previous meta-analysis (Wang et al., 2018) suggests a decrease in microbially derived binding agents. N addition decreased the proportion of the silt-clay fraction perhaps because N addition can reduce the production of microbially derived agents. In addition, a recent study reported that N-induced soil acidification is likely to decrease microaggregation (Ye et al., 2018). The latter authors proposed that declining soil pH increased the leaching of metal cations along with nitrate, which impeded the formation of microaggregates by reducing persistent agents. Unfortunately, although our meta-analysis indicated that N addition significantly decreased soil pH (Table 1), we did not find a significant relationship between the response ratios of microaggregates and soil pH.

4.2 | N enrichment increased the C concentration in soil aggregates

Although exogenous N addition in previous studies substantially increased the input of aboveground C into soil, N enrichment caused only small increases in the soil C reservoir, especially in the mineral soil layer (Chen et al., 2015; Liu & Greaver, 2010; Lu et al., 2011). Deng et al. (2018) reported that N enrichment increased the concentration of SOC by only 2% in China, which is similar to the effect of N enrichment on bulk soil C (+5%) in the current study (Figure 2). Whether N enrichment enhances C storage in soil is determined by the balance of aboveground C inputs and belowground C decomposition. A potential explanation for the uncoupled aboveground–belowground C dynamics is that even though N enrichment can increase the input of organic matter into soil, most of the input may be lost because of soil leaching and microbial respiration (Liu & Greaver, 2010). Previous meta-analyses found that N enrichment significantly increased the concentration of dissolved organic C in soil in the terrestrial ecosystems of China (Chen et al., 2015; Deng et al., 2018), which suggests that N enrichment increases the quantity of soil C available for leaching. Furthermore, even though the effect of N addition on microbial communities differs among previous meta-analysis studies (Wang et al., 2018; Zhou et al., 2017), N enrichment did stimulate the degradation of simple compounds in soil by increasing hydrolytic enzyme activities (Jia et al., 2020; Jian et al., 2016; Waldrop et al., 2004), which is consistent with the effects of N addition on cellulase and β-1,4-glucosidase in our meta-analysis (Table 1).

According to our meta-analysis, N enrichment notably increased the concentration of C in macroaggregates but had minor effects on the concentrations of microaggregate C and silt-clay C (Figure 2). In other words, N enrichment had similar effects on soil aggregate C and on soil aggregation. The organic matter in litter residues and soil particles are bound together by microbial binding agents to form stable soil aggregates that protect the organic compounds in the aggregates from microbial decomposition (Blanco-Canqui & Lal, 2004). The positive effect of N addition on the C concentration is greater for macroaggregates than for the bulk soil (Figure 2), which reflects the physical protection of C in soil aggregates (Blanco-Canqui & Lal, 2004). According to previous studies, macroaggregates are mainly formed by litter residue and microaggregates are formed within macroaggregates, which then become POC, while silt-clay fraction is related to MOC accumulation (Blanco-Canqui & Lal, 2004; Six et al., 2000). The effect of N addition on the SOC in organic horizon (+17%, Liu & Greaver, 2010; +3.8%, Chen et al., 2015) is similar to the effect of N addition on macroaggregate C, while the effect of N addition on the SOC in the mineral soil horizon (unaltered, Chen et al., 2015; Liu & Greaver, 2010; Lu et al., 2011) is similar to the effect of N addition
on silt-clay C. The response of SOC to N enrichment in soil aggregates differs from that of bulk soil C, and recognition of the contrasting responses of the POC pool and the MOC pool to N enrichment should increase our understanding of the responses of the whole soil C pool to N enrichment.

Abiotic factors, such as soil pH (Figure 5a,e) and ambient conditions (i.e., MAP and MAT, Table S2), were associated with soil aggregate C sequestration. The response ratio of macro- and microaggregate C increased linearly with decreasing soil pH, which is consistent with the acidity model established by Averill and Waring (2018). N-induced soil acidification suppresses the decomposition of organic matter by limiting the growth of microbial biomass, and thereby increases the accumulation of POC but suppresses the accumulation of MOC. In addition, MAT and MAP had pronounced effects on macroaggregate C and silt-clay C (Table S2), indicating that water and temperature help determine soil C dynamics. Wang et al. (2015) reported that addition of high volumes of water accelerated the combining of litter residue and soil organic matter and increased the formation of macroaggregates in a semiarid grassland. High MAP may enhance soil C sequestration by improving soil structure (He et al., 2018). In two previous studies, the effects of N deposition on litter decomposition and microbial growth were influenced by MAT and MAP (Deng et al., 2018; Zhang et al., 2018), resulting in variable effects on soil aggregate C.

The response ratio of macroaggregate C and the macroaggregate C sequestration decreased linearly with MBC (Figure 5c,d), suggesting that POC accumulation was affected by microbial C utilization. Multiple biological suppression theories, which are major associated with the inhibition of oxidase enzymes, have been proposed to explain the reduction of soil C decomposition under N enrichment (Bonner et al., 2019; Fog, 1988; Waldrop et al., 2004). The inhibition of oxidase activity is thought to reduce the microbial degradation of recalcitrant compounds such as lignin. van Diepen et al. (2015) reported that ligninolytic enzyme activities significantly decreased by N addition, and hence reinforce the inhibition effects of N enrichment on litter decay, particularly for high lignin species. Our meta-analysis indicated that N addition significantly decreased the activity of peroxidase (Table 1), which was consistent with the results of previous meta-analyses (Jia et al., 2020; Jian et al., 2016). Consequently, the inhibition of oxidase enzymes by N addition increases the quantity of plant residues available to form macroaggregates and increases the POC pool. N addition, in contrast, limits microbial C utilization, resulting in a reduction of the MOC pool.

4.3 Effects of ecosystem types and fertilization regimes

The effects of N deposition on C sequestration in soil aggregates have been widely investigated in different terrestrial ecosystems (Chang et al., 2019; Chen et al., 2019; Ye et al., 2018; Zhong et al., 2017). N addition significantly increased the macroaggregate C in natural ecosystems (subtropical/temperate forests and grasslands) but had a minor effect on it in managed ecosystems (croplands; Figure 4a). Previous studies reported that tillage greatly affects the stability of soil aggregates (Degryze et al., 2004; Oades, 1984). Tillage can damage roots, reduce microbial population, and cause the release of organic binding agents, which is expected to increase the decomposition of organic residues (Six et al., 2000), and thus reducing macroaggregate C (Blanco-Canqui & Lal, 2004). The different responses of macroaggregates to N enrichment in natural versus managed ecosystems suggest that tillage may reduce the macroaggregation, thereby mask the positive effects of N addition on macroaggregate C sequestration. In the current study, the effect of N addition on soil aggregates C was greater for temperate forests than for subtropical forests (Figure 4). We attribute it to a generally high level of ambient N deposition in the subtropical region of China (Liu et al., 2013; Zhu et al., 2015); thus, the effect of exogenous N addition on subtropical forests may be relatively insensitive compared to N-limited temperate forests. Unexpectedly, N enrichment had small effects on the soil aggregation among different ecosystem types (Figure 3). The general linear model indicated that MAT and MAP did not affect soil aggregation (Table S2), which suggested that ambient conditions were not the major factors affecting soil structure.

Organic N fertilizer and inorganic N fertilizer had inconsistent effects on soil aggregation and the soil aggregate C (Figures 3 and 4), indicating that N fertilizer form is important in soil C sequestration. The effects of N addition on woody biomass increase (Schulte-Uebbing & de Vries, 2018; Yan et al., 2019), organic matter decomposition (Chen et al., 2015), and microbial biomass (Jia et al., 2020; Wang et al., 2018) differed depending on N fertilizer form, which helps explain the effects of fertilizer form on soil aggregates. In previous studies, increases in plant biomass were significantly higher with urea than with NH$_4$NO$_3$ (Schulte-Uebbing & de Vries, 2018; Yan et al., 2019), leading to a greater input of plant residue into soil with urea. The increasing fresh residue enhances the formation of large diameter aggregates (Six et al., 2000) and organic matter input into soil. This explains why the MWD and macroaggregate C were significantly higher with organic fertilizer than with inorganic fertilizer in our meta-analysis. Furthermore, organic N fertilizer increased soil microbial biomass more than inorganic fertilizer (Jia et al., 2020). The different effect sizes of N addition via organic versus inorganic fertilizer on MBC (Figure S2) provide further evidence that soil microorganisms are more positively affected by organic fertilizer. Thus, the silt-clay C concentration was significantly higher with organic fertilizer than with inorganic fertilizer.

The effect sizes of N addition on macroaggregate C and bulk soil C were significantly affected by experimental duration (Table S1), that is, the concentrations of macroaggregate C was significantly increased with short- and long-term N addition, whereas bulk soil C marked increased with medium-term N addition (Figure 4a,d). These effects require further research, however, because the results of the current meta-analysis were inconsistent with those of a previous meta-analysis (Liu & Greaver, 2010). Experimental duration has been considered an important factor that influences the effect of N addition on soil microbial communities and plant growth. For instance,
several field experiments indicated that short-term N addition significantly decreased MBC and that this effect disappeared as the experimental duration increased (Liu et al., 2013; Tian et al., 2020). Ten years of N-addition experiment revealed that plants maintained a foliar nutrient supply by altering their water-use strategy under long-term, high N deposition (Lu et al., 2018). Previous meta-analyses found that the responses of microbial communities and litter decomposition to N addition depend on experimental duration (Wang et al., 2018; Zhang et al., 2018). The gradual adaptation of soil microbial communities and plants to N enrichment may influence the response of soil C sequestration to N enrichment. According to our results, despite the substantial increases in POC via N enrichment, enhancement of SOC might gradually fall back as the rebalance of belowground carbon-related process at long-term N deposition.

4.4 | Limitations and implications

Our meta-analysis assessed the responses of soil aggregates to N enrichment at a large scale. We found that N enrichment increased soil aggregation and C storage in terrestrial ecosystems in China. Based on the results, we present a conceptual model that explains the response of soil aggregates to N enrichment (Figure 6) and that illustrates the potential mechanisms that cause an increase in the soil C sequestration with N deposition.

Our study has several limitations. First, N was applied directly to soil, rather than to forest canopy as atmospheric N deposition, in most forest N-addition experiments. Understory N-addition experiment overlooks the absorption, retention, and transformation of N by forest canopy and thus may overestimate the effect of atmospheric N addition on forest soils (Lu et al., 2021). Recent canopy N-addition studies suggest that canopy N addition and understory N addition have different effects on tree growth (Tang et al., 2020), soil microbial properties (Liu et al., 2020), and soil C dynamics (Lu et al., 2021). These findings have implications for future research to examine the potential effects of forest canopies in simulated N deposition experiments. Second, the experimental duration of most of the collected studies was less than 10 years, and only 24 observations of soil aggregate C concentration were collected from long-term N-addition treatments in subtropical forests. This limited our ability to accurately compare the long-term effects of N enrichment on soil aggregate C concentration (especially in the MOC pool) among ecosystems (Averill & Waring, 2018; Ye et al., 2018). More data from long-term field experiments are needed to better understand the effect of chronic N enrichment on soil C pool. Finally, we acknowledge that atmospheric N deposition is a chronic, low-dose input of N into ecosystems, whereas many N fertilization experiments use single, annual pulses. In particular, studies that explicitly manipulate the frequency of N addition find that responses to N enrichment are much weaker when N is applied as many doses over the course of the year, rather than as a single addition (Cao et al., 2019). This finding suggests that ecosystems respond very differently to chronic, low-dose versus high-pulse dose input of N. It is preferable that future studies could document similar patterns over N deposition gradients.

In summary, our meta-analysis indicated that input of exogenous N promoted soil C sequestration in terrestrial ecosystems in China. Soil aggregates are important in the soil C dynamics induced by anthropogenic N emission. Different responses of organic and mineral soil layer to N deposition have been previously reported (Chen et al., 2015; Liu & Greaver, 2010; Lu et al., 2011). The competing hypotheses on this topic indicate sharply contrasting views of N-induced soil C sequestration. We feel the different changes of soil aggregate C will add greater clarity in predicting soil C pool accumulation.

**FIGURE 6** Conceptual model of the dynamic responses of soil aggregates to N enrichment and the potential mechanisms involved in soil aggregation and C sequestration. According to this model, N enrichment substantially increases the input of organic matter to soil by increasing soil N availability, promoting macroaggregation, and protecting particulate organic C against microbial decomposition. N-induced soil acidification can further inhibit microbial degradation by decreasing the activity of oxidase enzymes. N enrichment decreases the proportion of silt-clay fraction which is related to microbial production, resulting in a limitation of mineral-associated organic C sequestration. The response of bulk soil C depends on the combined effects of individual aggregate C, and generally shows a slight increase by N enrichment. The blue, red, and black boxes indicate variables that are positively, negatively, or not changed by N enrichment. ‡ result refers to Deng et al. (2018) and Lu et al. (2011) [Colour figure can be viewed at wileyonlinelibrary.com]
responses to N fertilization. Enhancement of aboveground C input in response to N enhancement significantly increases macroaggregate C and therefore increases the POC pool in the organic soil layer. In the mineral soil layer, however, N enhancement had a minor effect on silt-clay C, which leads to no significant change in the MOC pool. The effects of N enrichment on soil C dynamics are highly variable among ecosystem types and depend on fertilization regimes. These findings suggest that multiple mechanisms control soil C sequestration in response to N enrichment. The general response of soil C pools to N enrichment is driven by the combined effects of individual aggregate C.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available at Figshare (https://doi.org/10.6084/m9.figshare.13646672.v1).

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