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Construction of anthropogenic cultivable soil: carbon-nitrogen coupling and microbial regulatory mechanisms

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Abstract

Despite the growing interest in anthropogenic soils, the mechanisms by which nitrogen enrichment positively contributes to anthropogenic soil organic carbon (SOC) pools and soil quality improvement remain unclear. This study investigated the driving mechanisms of nitrogen addition on the turnover of soil aggregates. Changes in soil carbon fractions and microbial communities following soybean cultivation were also analyzed. The results revealed that the contents of SOC and various carbon fractions in anthropogenic soil aggregates was significantly higher than those in weak soil aggregates. The predominant breakdown pathway of anthropogenic soil aggregates was the transition from > 2 mm to 0.25-2 mm, whereas the primary formation pathway were 0.053–0.25 mm to 0.25-2 mm. The aggregate formation rate reached its maximum at a nitrogen application rate of 120 kg/ha. Nitrogen addition significantly increased the contents of active carbon fractions in soil aggregates, while exerting no significant effect on the content of inert carbon fractions. Optimal nitrogen addition increased the relative abundances of *Bacteroidota*, *Actinomycetota* and *Basidiomycota*, thereby establishing a favorable microbial microenvironment to enhance soil quality and crop yield. Active soil carbon fractions, soil aggregate stability, and nitrogen addition rate were significantly and positively correlated with soybean yield. Moderate nitrogen addition to anthropogenic soil significantly promoted soybean root architecture development and improved soybean quality and yield. This study can contribute to the understanding of soil carbon dynamics and provides a theoretical basis for enhancing soil carbon sequestration capacity and improving soybean yield.

1. INTRODUCTION

- With rapid economic development and global population growth, food security has become one of the most pressing challenges for sustainable social development. Soybean (Glycine max L.), a vital global food and oilseed crop, has experienced stagnating or declining yields in recent years due to widespread soil degradation [1]. Soil organic carbon (SOC) represents the largest terrestrial carbon pool. As a heterogeneous continuum composed of diverse functional components, SOC lies at the core of soil quality, and its dynamic regulation of soil health is a critical determinant of global food and nutritional security [2]. Consequently, the mechanisms governing SOC formation, stabilization, and preservation under global environmental change have received widespread attention. Due to the inherent complexity of SOC, increasing evidence indicates that treating it as a single, homogeneous pool is insufficient; rather, partitioning SOC into distinct functional fractions is essential for elucidating and predicting its dynamics and responses to environmental perturbations [3]. SOC is conventionally categorized into active fractions—such as light fraction organic carbon (LOC), readily oxidizable carbon (ROC), and particulate organic carbon (POC)—and inert fractions, including heavy fraction organic carbon (HOC), recalcitrant organic carbon (NOC), and mineral-associated organic carbon (MAOC). Each of these fractions possesses distinct physicochemical properties and turnover times, fulfilling complementary roles within soil ecosystems [4, 5].

Soil aggregates are the fundamental structural units of soil and serve as crucial indicators of soil quality and agroecosystem health. Their stability and particle-size distribution directly govern the physical protection, migration, and fractional transformation of SOC [6, 7]. The fragmentation and turnover dynamics of soil aggregates profoundly influence overall structural stability. It is generally recognized that macroaggregates (> 0.25 mm) occlude greater amounts of fresh, labile, and decomposable organic carbon and account for a higher mass proportion, thereby exhibiting a strong capacity for carbon sequestration [8]. Macroaggregates also feature a well-developed pore network, which facilitates water infiltration and oxygen diffusion, thereby stimulating microbial activity and metabolism [9]. Conversely, the organic carbon associated with microaggregates (< 0.25 mm) is predominantly highly aromatic, exhibits a longer residence time, and is less accessible to microbial decomposition, which promotes the long-term stabilization of SOC [10]. Therefore, clarifying the composition of soil organic carbon, the fragmentation and turnover processes of soil aggregates, and their stability is crucial for field carbon management and sustainable agricultural development.

Nitrogen (N) is a primary limiting element in terrestrial nutrient cycling and exerts a profound influence on agroecosystems. In nitrogen-limited agroecosystems, nitrogen fertilizer inputs are necessary to maintain soil fertility to ensure crop yields [11]. This is particularly true in soybean cultivation, where the amount of nitrogen fixed by symbiotic nitrogen-fixing bacteria does not fully meet the soybean's growth and development needs, making the application of additional nitrogen necessary [12]. Harmonizing exogenous N supply with biological nitrogen fixation in root nodules is crucial for optimizing both the seed quality and yield of soybeans. In addition, since the carbon and nitrogen cycles are tightly coupled, nitrogen addition will lead to changes in the feedback of the carbon cycle to climate change. Research has confirmed that the addition of nitrogen typically increase plant photosynthetic capacity, stimulate plant growth, and result in increased plant carbon stocks [13]. However, the net effects of nitrogen addition on subsurface SOC stocks and fractional composition remain controversial. Some studies have demonstrated that nitrogen addition positively impacts soil structural stability, with nitrogen increasing the formation of macroaggregates and reducing the decomposition of reactive organic carbon [14]. Nevertheless, other studies have produced conflicting results, indicating that nitrogen addition can disrupt soil aggregate structure and accelerate the decomposition of reactive organic carbon [15]. These discrepancies suggest that the impacts of nitrogen addition on soil aggregation and carbon fractions are complex and context-dependent.

Our team developed a method for producing artificial humus from lignocellulosic waste, which was recognized among the IUPAC Top Ten Emerging Technologies in Chemistry 2021. This technology enables the rapid conversion of agricultural waste into artificial humus within 24 hours, achieving an organic matter content exceeding 80% and a humification degree above 70%. Building on this foundation, we have innovatively proposed the hydrothermal humification-hydrothermal carbonization (HTH-HTC) technology. This technology plays a significant role in the remediation of degraded soils. In our prior work, constructing anthropogenic soil via HTH-HTC significantly elevated soil organic matter content and fertility, improved physical structure, and reshaped bacterial and fungal community compositions; specifically, beneficial taxa involved in nutrient cycling and plant growth promotion

proliferated, enabling the reconstruction of a robust soil microbiome within a short maturation period (28 days) [16–19]. Nevertheless, how exogenous nitrogen addition interacts with HTH-HTC-constructed anthropogenic soil to regulate aggregate turnover, SOC fractionation, and soybean productivity remains unclear. Therefore, this study aimed to: (1) elucidate the driving mechanisms of nitrogen addition on the breakdown and formation pathways of anthropogenic soil aggregates using rare earth oxide (REO) tracers; and (2) evaluate the responses of aggregate stability, SOC fractions, microbial community structure, and soybean yield to varying nitrogen application rates.

2. MATERIALS and METHODS

2.1. Experiment design

The original weak soil used in this study was obtained from Northeast Agriculture University in Harbin, China (45º44'22”N, 126º43'30”E). This original weak soil is classified as degraded black soil with extremely low organic matter content, and other fundamental properties as shown in Table 1. The anthropogenic soil was fabricated by processing a mixture of degraded soil and crop straw powder via HTH-HTC technology, followed by blending the resulting hydrochar–humic product with the original degraded soil and incubating it to maturity. To investigate aggregate fragmentation and turnover dynamics, four nitrogen application rates, N1 (0 kg N/ha), N2 (60 kg N/ha), N3 (120 kg N/ha), and N4 (240 kg N/ha)—were applied to both degraded (D1) and anthropogenic (A1) soils, which were then incubated for 112 days. Subsequently, a pot experiment with soybean (Glycine max L.) was conducted using the anthropogenic soil, with the degraded soil serving as the control. The planting period was from May to September 2025 at Northeast Agricultural University in Harbin, Heilongjiang Province. Potassium dihydrogen phosphate and urea were applied as the basal fertilizer before planting. The potassium dihydrogen phosphate application rate was 80 kg/ha, while nitrogen application rates are N1, N2, N3, and N4. Ten kilograms of degraded or anthropogenic soil were placed into each pot, establishing eight treatment combinations: D1N1, D1N2, D1N3, D1N4, A1N1, A1N2, A1N3, and A1N4. Each treatment was replicated in three pots, and soil moisture was adjusted to optimal field capacity prior to sowing. The soybean variety selected for this study was 'Hei Nong 48'. High-quality seeds were selected, ensuring they were free from insect damage, mould, deflation and breakage. The seeds were dried in the sun for 6 hours before sowing, followed by immersion for 12 hours. Five soybeans were placed in each pot and sown at a depth of 2–3 cm. Water regularly after sowing. When the soybeans are mature, record protein content, oil content, number of pods per plant, number of grains per plant, weight per plant, hundred-grain weight and theoretical yield. The particle size distribution and stability of soil aggregates, soil carbon fraction and soil microbial community were analyzed.

Table 1
Basic indicators of weak soil.

	pH	TOC (%)	CEC (cmol(+)/kg)	Total nitrogen (g/kg)	Total phosphorus (g/kg)
Weak soil	6.48	0.769	25.39	1.96	2.37

2.2. Analysis of soil aggregate stability and turnover

A wet-sieving method was employed to fractionate water-stable soil aggregates [20] to obtain the aggregates > 2 mm, 1–2 mm, 0.5-1 mm, 0.25–0.5 mm, 0.053–0.25 mm and < 0.053 mm. The wet sieving method is described in Text S1. To further characterize the stability of the soil aggregates, two additional parameters were collected: mean weight diameter (MWD) and the proportion of water-stable soil aggregates > 0.25 mm ($R_{0.25}$).

$$\mathrm{MWD}=\sum_{i=1}^{n} x_i w_i$$

1

where *xi* (mm) is the average diameter, *wi*(g) is the weight fraction in each class.

The rare earth oxide tracer method was employed to investigate the turnover process of aggregates. REOs were used to label the aggregates, gadolinium oxide (Gd_2O_3), lanthanum oxide (La_2O_3), samarium oxide (Sm_2O_3), and neodymium oxide (Nd_2O_3) were used to label aggregates measuring < 0.053 mm, 0.053–0.25 mm, 0.25-2 mm, and > 2 mm, respectively [21]. The purity of each oxide was > 99.9%. Inductively coupled plasma mass spectrometry (ICP-MS) was employed to determine the REO concentrations in each size fraction following incubation, enabling the calculation of aggregate breakdown and formation rates [22]. Further details of the labeling and calculation protocols are provided in Text S2.

2.3. Determination of soil carbon fractions

TOC: Soil TOC content was determined using a total organic carbon analyser (Shimadzu, Japan). ROC and NOC: soil ROC was determined using 333 mmol/L $KMnO_4$ and colorimetrically analyzed on a spectrophotometer at 565 nm. NOC is the difference between SOC and ROC [23]. MAOC and POC: POC and MAOC were obtained by adding a 0.5% $(NaPO_3)_6$ solution (30 mL) to a 10 g soil sample, shaking at 200 rpm for 18 hours, and then filtering the sample through a 0.053 mm sieve. The material that passed through the sieve was considered to be MAOC, while the material that remained on the sieve was considered to be POC [24]. LOC and HOC: Soil LOC and HOC were determined by density fractionation [25]. Text S3 describes the detailed method.

2.4. Analysis of soil microbial diversity

High-throughput amplicon sequencing was performed to evaluate the effects of nitrogen addition on soil microbial community structure. Post-harvest soil samples (5 g per treatment group) were immediately flash-frozen and stored at -80ºC prior to DNA extraction and bacterial and fungal community profiling. Alpha diversity is a common measure of the abundance and diversity of bacterial and fungal communities in soil. The specific operational procedure is shown in Text S4. All samples were sent to Shanghai Majorbio Biomedical Technology Co. for high-throughput sequencing, and each sample was tested in triplicate. The data were analyzed on the free online platform of Majorbio Cloud Platform (www.Majorbio.com).

2.5. Determination of soybean quality and yield

The IN-GX02 root scanner was used to observe the development of the soybean roots, after which the actual yield of each group of soybeans was calculated. A FOSS Infratec 1241 near infrared grain analyzer was used to determine the protein and oil content of soybean seeds [26, 27]. Text S5 provides a detailed description of the method. At the point of soybean grain maturity, five representative plants were selected from each group in order to measure the following yield components: number of pods per plant, number of grains per plant, weight per plant, hundred-grain weight. These agronomic traits were assessed via weighing and manual counting. Following threshing, the grain was air-dried and weighed to calculate theoretical yield.

2.6. Correlation and statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 19.0. All data are expressed as the mean $\pm$ standard deviation (SD) of at least three independent replicates, and one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$) was used to determine significant differences among treatments. Figures were generated using Origin 2022. Relationships among soil aggregate stability, carbon fractions, nitrogen application rates, and soybean yield were evaluated by calculating Pearson’s correlation coefficients (r) using the Correlation Plot app in Origin 2022.

3. RESULTS

3.1 Turnover of nitrogen-rich anthropogenic soil aggregates

As shown in Figure S1, there were six breakdown paths and six formation paths for the turnover of the four aggregates. The turnover paths of the aggregates were calculated from the rare earth oxides (REOs) of each particle size aggregate, the results can be seen from Fig. 1 and Figure S2. The calculation process required determining the background values of REOs in each group, the results of which are shown in Table S3. The total value of the breakdown pathway was greatest for aggregates measuring > 2 mm in size, in anthropogenic soil and weak soils, with the main turnover pathway being the breakdown of aggregate size from > 2 mm to 0.25-2 mm. The total value of the formation pathway was found to be most significant for 0.25-2 mm aggregates, with the primary turnover pathway being the formation of

aggregate size from 0.053–0.25 mm to 0.25-2 mm. As the incubation time increased, there was a concomitant decrease in the degree of aggregate breakdown and an increase in the intensity of aggregate formation. The degree of aggregate breakdown decreased from 13.93%-51.36% to 7.84%-43.96%, while the formation pathways increased from 0.27%-41.69% to 0.41%-57.15% in the anthropogenic soil compared to the weak soil. Furthermore, the addition of nitrogen significantly affected the breakdown and formation of soil aggregates. The weakest breakdown and the strongest formation of aggregates were observed at a nitrogen addition concentration of 120 kg/ha. The breakdown values of D1N3 ranged from 13.96% to 48.75% and the formation values ranged from 0.49% to 41.69%. By contrast, the breakdown values of A1N3 ranged from 7.24% to 23.03% and the formation values ranged from 1.09% to 57.15%.

3.2 Soils aggregate distribution and water stability

As shown in Fig. 2, the particle size distribution and stability of soil aggregates were observed in each treatment group following soybean planting. In weak soils with varying nitrogen concentrations (D1N1, D1N2, D1N3 and D1N4), the highest percentage of aggregates was founded to be in the range of 0.053–0.25 mm (63.75%, 71.09%, 57.52%, and 61.47%), while the lowest percentage of aggregates was founded to be in the range of 0.5-1 mm (1.44%, 1.35%, 2.63% and 1.00%). Furthermore, the proportion of aggregates measuring between 1–2 mm and > 2 mm was also found to be minimal. Anthropogenic soils with added nitrogen at different concentrations (A1N1, A1N2, A1N3 and A1N4) had the highest percentage of aggregates > 2 mm in the total particle size, 56.92%, 64.67%, 66.67% and 60.40%. The lowest percentage of aggregates was found to be between 0.25–0.5 mm, at 1.83%, 1.80%, 2.17% and 2.47%, respectively. MWD and $R_{0.25}$ are pivotal indicators for the characterization of aggregate stability [28]. An increase in both MWD and $R_{0.25}$ indicates a higher proportion of large aggregates in the soil and, consequently, a higher degree of stability. As shown in Fig. 2, the MWDs of D1N1, D1N2, D1N3, and D1N4 were measured to be 0.2473 mm, 0.4928 mm, 0.5259 mm, and 0.3789 mm, with the $R_{0.25}$ values recorded as 10.19%, 13.02%, 17.12%, and 10.61%, respectively. The MWDs of A1N1, A1N2, A1N3, and A1N4 were 3.4792 mm, 3.9788 mm, 4.0911 mm and 3.7498 mm, the $R_{0.25}$ values were 62.33%, 71.36%, 74.72%, and 71.61% respectively.

Note

Different letters in the graph represent significant differences ($p < 0.05$).

3.3 Soil organic carbon content in soil aggregates

It is widely acknowledged that soil aggregates play a pivotal role in stabilizing SOC, which has been demonstrated to be a critical factor in preserving soil structural stability. Soil aggregate organic carbon content in each treatment group post-planting of was shown in Fig. 3. Firstly, an increase in the SOC at all aggregate level in the anthropogenic soil treatment group was observed when compared with the weak soil. However, the fixation of SOC by aggregates in anthropogenic soil and weak soil was found to

be primarily concentrated in aggregates > 0.25 mm. In weak soils, the highest carbon content was found in 1–2 mm aggregates with 4.62% (D1N2), and the lowest carbon content was found in aggregates < 0.053 mm with 0.33% (D1N4). In anthropogenic soils, the highest carbon content was found in aggregates of 0.5-1 mm with 6.67% (A1N2), and the lowest was found in aggregates < 0.053 mm with 1.12% (A1N4). Secondly, compared with soil without added nitrogen, the organic carbon content of soil large-size aggregates exhibited an increase with added nitrogen concentrations of N2 = 60 kg/ha and N3 = 120 kg/ha. However, the accumulation of aggregate organic carbon was found to be unfavorable at a nitrogen concentration of N4 = 180 kg/ha.

Note

Different letters in the graph represent significant differences ($p < 0.05$).

3.4 Organic carbon content of fractions in soil aggregates

Different carbon fractions have been shown to exhibit divergent patterns and turnover times, and to be more sensitive to environmental changes than total organic carbon. As depicted in Figure S3 and Figure S4, MAOC, NOC and HOC accounted for a higher proportion in the weak soil. The addition of nitrogen had no significant effect on enhancing MAOC and NOC in weak soil, but significantly increased the proportion of LOC, which was highest in the D1N3 group at 18.21%. The percentage of POC, NOC and HOC in anthropogenic soil was high and the addition of nitrogen significantly increased the percentages of LOC and POC. The highest percentages were 16.26% for LOC in the A1N3 group and 58.30% for POC in the A1N4 group. The effect on NOC, however, was not significant. In terms of carbon content, anthropogenic and weak soils exhibit the same pattern: MAOC, NOC, and HOC have the highest carbon content, and the effect of nitrogen addition is not significant.

Figure 4 and Fig. 5 analyse the distribution of each SOC component in different aggregate grain sizes of weak and anthropogenic soils. The carbon content of each component was found to be higher in the anthropogenic soil than in the weak soil. The highest carbon content of each component was found in aggregates of 0.5-1 mm, 1–2 mm and > 2 mm in the weak soil, while the carbon content in the anthropogenic soil was mainly concentrated in the three grain sizes of 0.25–0.5 mm, 0.5-1 mm, and 1–2 mm. Nitrogen addition significantly affected the carbon content of LOC, ROC and POC, but the enhancement effect was less obvious when nitrogen addition was excessive. The carbon content of NOC, MAOC, and HOC exhibited minimal sensitivity to nitrogen addition.

Note

Different letters in the graph represent significant differences ($p < 0.05$).

Note

Different letters in the graph represent significant differences ($p < 0.05$).

3.5 Composition of soil microbial communities

Soil microorganisms have been shown to play a crucial role in the soil and have important effect on the stability of soil structure, the formation and turnover of soil aggregates, and enhancing the capacity for soil carbon sequestration. The composition of the microbial communities of four representative groups (D1N1, D1N3, A1N1 and A1N3) was analysed, results are shown in Fig. 6.

In weak soil, the dominant bacterial phyla were *Pseudomonadota* (38.47%-39.32%), *Actinomycetota* (13.42%-18.41%), *Bacteroidota* (6.32%-9.52%), *Bacillota* (10.28%-12.38%). In anthropogenic soil. The most dominant bacterial phyla in anthropogenic soil were *Pseudomonadota* (34.35%-40.81%), *Actinomycetota* (14.92%-18.48%), *Bacteroidota* (14.31%-14.81%), and *Bacillota* (4.38%-9.85%). Anthropogenic soil showed a significant increase in *Bacteroidota*, *Actinomycetota*, and *Patescibacteriota* in comparison to the weak soil. The addition of nitrogen promoted the increase in the number of *Bacteroidota*, *Actinomycetota* in the weak and anthropogenic soils (Fig. 6a). As shown in Fig. 6b, the predominant fungal phyla in weak soils were *Ascomycota* (61.45%-92.34%) and *Basidiomycota* (2.64%-4.29%), while in anthropogenic soils were *Ascomycota* (17.91%-32.84%) and *Basidiomycota* (67.15%-82.05%). The number of *Basidiomycota* in anthropogenic soil was higher than in the original soil. In contrast, the *Ascomycota* content exhibited a substantial decline.

Alpha diversity analysis is a common method of measuring the abundance and diversity of bacterial and fungal communities in soil. Here, the Chao1 and ACE indices were used to evaluate the richness of each soil sample, and the Shannon and Simpson indices were used to evaluate its diversity. The indices for each sample are shown in Table S4 and Table S5. In terms of soil bacterial abundance, Chao1 index ranked as A1N3 > D1N1 > A1N1 > D1N3, with the ACE index ranked as A1N3 > D1N1 > A1N1 > D1N3. And there is no significant difference between the Shannon index and Simpson index of the four groups. From a fungal perspective, the Chao1 index ranked as follows: A1N3 > A1N1 > D1N3 > D1N1; the ACE index ranked as follows: A1N3 > A1N1 > D1N3 > D1N1. The Shannon and Simpson indices for the anthropogenic soil groups were also higher than those for the weak soil groups.

3.6 Soybean quality and yield

The cultivation of soybean was undertaken in both weak and anthropogenic soils. The flowering and pod formation stage represents the most vigorous period of soybean growth, requiring the greatest amount of water and nutrients. This phase is crucial for both yield and quality. We documented the growth and development of different treatment groups during the flowering and pod formation stage, as illustrated in Fig. 7. Key indicators such as plant height and number of leaves were significantly higher in the anthropogenic soils group compared to the poor soils group. Furthermore, soybean growth was markedly superior under nitrogen additions of N2 and N3 relative to N1 and N4.

Note

Different letters in the graph represent significant differences ($p < 0.05$).

As can be seen from the above analyses, soil aggregate stability, soil carbon fraction and nitrogen addition are closely related to soybean yield. As shown in Fig. 9, there is a strong correlation between the main key factors and soybean yield. The analysis showed that soybean yield was positively correlated with nitrogen addition, MWD, $R_{0.25}$, LOC, ROC, and POC. Soybean yield showed a more significant positive correlation with 0.053–0.25 mm LOC, 0.25–0.5 mm LOC, < 0.053 mm ROC, 0.25–0.5 mm ROC, 0.5-1 mm ROC, and 1–2 mm ROC all showed a more significant positive correlation ($0.01 < p < 0.05$). MWD showed a significant positive correlation with $R_{0.25}$ ($p < 0.001$), and both MWD and $R_{0.25}$ showed a positive correlation with both ROC and LOC ($p < 0.001$).

4. DISCUSSION

4.1 Effects of nitrogen addition on soil aggregate turnover and stability

The aggregate formation rate in the anthropogenic soil was substantially higher than that in the weak soil. Across treatments, aggregate breakdown predominantly proceeded from the > 2 mm fraction into the 0.25-2 mm fraction, whereas aggregate formation primarily occurred via the assembly of 0.053–0.25 mm microaggregates into 0.25-2 mm macroaggregates. Nitrogen fertilization promoted macroaggregate formation, with formation pathway rates increasing progressively up to an application rate of 120 kg/ha (N3), where aggregate formation peaked. Furthermore, soil reconstruction via HTH-HTC technology markedly elevated the mass proportion of macroaggregates and enhanced overall water stability (Fig. 2). During the HTH-HTC and subsequent soil maturation processes, artificial humic substances and hydrochar act as core binding agents that bridge mineral particles and drive the hierarchical assembly of microaggregates into macroaggregates [18]. Previous studies have also shown that adding exogenous organic matter stimulates microbial function and diversity and that accumulating organically bound material on the soil surface promotes the formation of large aggregates [29, 30]. This is one reason for the higher aggregate formation pathway values in anthropogenic soils than in weak soils, and a key factor in the significant increase in the proportion of macroaggregates in anthropogenic soils.

Nitrogen plays a crucial role in the formation and stability of soil aggregates, and the quantity applied is particularly important. Adding nitrogen at concentrations of N2 and N3 has a positive effect on the stability of soil structure and can increase the formation of macroaggregates. This is because nitrogen addition affects the production of cementing substances, promotes the combination of mycelium and soil particles to form macroaggregates and improves the environment of the soil microbial community, promoting the growth of beneficial bacteria and accelerating the formation of aggregates [31–33]. However, excessive nitrogen addition (N4 = 240 kg/ha) is not conducive to the formation of macroaggregates because it is likely to cause soil crusting and destroy the soil structure. It is also likely to cause an imbalance in soil acidity and alkalinity, thereby destroying the soil microbial environment

[34]. Therefore, the key to promoting the stabilization and structural improvement of anthropogenic soil aggregates lies in the nitrogen application rate.

4.2 Effect of nitrogen addition on organic carbon and carbon fractions in aggregates

The production of anthropogenic humic matter, biochar and other products during the construction of anthropogenic soil using HTH-HTC technology increased the carbon content of anthropogenic soil. This resulted in significantly higher SOC and other carbon fractions in anthropogenic soil aggregates than in weak soil aggregates. The new organic carbon storage in the soil following nitrogen addition primarily occurred in the macroaggregates, demonstrating their pivotal role in storing and protecting SOC under nitrogen-enriched conditions. While macroaggregates cannot directly protect SOC in the long term, they can fix more of it. Additionally, high nitrogen levels (N4 = 240 kg/ha) destabilise the aggregates, thus inhibiting the accumulation of SOC. From the perspective of carbon fractions, the maximum content of ROC, LOC and POC in anthropogenic soil aggregates was 1.766%, 0.803% and 2.312% respectively, all of which showed significant increases compared to weak soil. Nitrogen addition significantly increased the content of active SOC, such as ROC, LOC and POC, in soil aggregates. This is consistent with the findings of Chen and Tang [35, 36]. After soybean harvest, soil carbon was mainly derived from inputs from the root system. Nitrogen addition increased total plant root biomass, with the increased root carbon directly raising the carbon fraction content. Furthermore, nitrogen addition promotes soybean growth, resulting in the transfer of photosynthetic carbon to the subsurface. Much of the input root carbon decomposes easily, thus increasing the carbon fraction content by promoting microbial decomposition [37]. Soil inert carbon is primarily stored in clay with a physically and chemically protected form, making it resistant to decomposition. In this study, NOC, HOC, and other inert carbon fractions were relatively stable and their content did not differ significantly under different nitrogen addition treatments.

4.3 Effect of nitrogen addition on soil microbial community structure and diversity

Changes in the microbial community in four representative soil groups (D1N1, D1N3, A1N1 and A1N3) were analysed. By analysing the taxonomic composition of the bacterial community in the soil, the dominant phyla in the anthropogenic and weak soils did not differ much, and all of them were *Pseudomonadota*, *Actinomycetota*, *Bacteroidota* and *Bacillota*. The bacterial community structure in the anthropogenic soil changed significantly compared with the weak soil, especially in terms of relative abundance. The abundance of *Bacteroidota*, *Actinomycetot*a, and *Patescibacteriota* increased significantly in the anthropogenic soil. Nitrogen addition promoted the increase in the abundance of *Bacteroidota* and *Actinomycetot*a. *Actinomycetota* promote aggregate formation by secreting extracellular polymers (EPS) such as polysaccharides and glycoproteins, which directly bind soil particles. In addition, *Actinomycetota* is also the main group of soil bacteria that degrade lignin, cellulose and other organic matter, and their decomposition products can be used as binders to encapsulate mineral particles and promote the turnover of microaggregates to macroaggregates [38–40]. It can also

work with *Pseudomonadota* and other bacteria to decompose organic matter, and the released cementing material and bacterial EPS together to build a stable three-dimensional network structure. *Bacteroidot*a is an important microbial group in soil, especially playing a key role in organic matter decomposition and soil structure formation [41]. The organic metabolites released during the decomposition of organic matter act as cementing agents, promoting the aggregation of soil particles and increasing the stability of aggregates through the metabolic activity of generating stable organo-mineral complexes [42]. During the decomposition of organic materials, *Bacteroidota* accelerates the turnover of organic carbon through carbon metabolism, which promotes the increase of TOC content and improves soil quality [43]. These findings suggest that anthropogenic soils with applied nitrogen have the potential to promote bacterial metabolism and provide a nutrient-rich environment, thereby improving soil quality and plant growth conditions. In our studies of microbial communities, we have paid particular attention to the taxonomic composition of fungi and how this varies.

The dominant fungal phyla in both anthropogenic and weak soils are *Ascomycota* and *Basidiomycota*. The amount of *Basidiomycota* was significantly increased in the anthropogenic soil compared to the weak soil. In contrast, the amount of *Ascomycota* decreased significantly. As an important taxon in the fungal community, *Basidiomycotas* play multiple key roles in soil ecosystems. *Basidiomycotas* are efficient decomposers, capable of degrading complex organic matter such as lignin and cellulose, accelerating the process of organic carbon mineralisation and promoting soil nutrient cycling [44, 45]. Through physical entanglement and secretion of cementing substances, the mycelium of *Basidiomycotas* promotes the formation of soil aggregates, enhances soil porosity and permeability [46], and certain *Basidiomycotas* inhibit the growth of plant pathogens through competition or antagonism, improving crop quality and yield [47]. Alpha diversity indices further confirmed that moderate nitrogen addition (N2 and N3) to anthropogenic soil maximized bacterial and fungal richness and diversity. This enriched and balanced microbiome accelerated aggregate stabilization, enhanced labile carbon retention, and ultimately underpinned superior soybean quality and yields.

4.4 Effect of nitrogen addition on quality and yield of soybean

The growth and development of soybeans, along with their quality and yield, serve as the optimal indicators for assessing soil quality. We analysed changes in the quality and yield of soybeans in anthropogenic and weak soils at different nitrogen addition levels and documented soybean growth during the flowering and pod formation period. Soybean protein content increased with nitrogen addition, while the difference in oil content was not significant. Soybean per plant weight, hundred grain weight and yield were higher in the anthropogenic soil group than in the weak soil group. The highest theoretical yield in the anthropogenic soil group was 7.82% higher than in the weak soil group. Soybean yield was positively correlated with soil aggregate stability, the active carbon fraction, and nitrogen addition. The excellent aggregate and pore structure of anthropogenic soil created a more favourable environment for root development and reduced root penetration resistance. The increased nutrients and improved air conditions directly promoted root system growth. The soybean root development diagram for

anthropogenic and weak soil planting is shown in Figure S5. The soybean root system in the anthropogenic soil group was denser and more robust, promoting nutrient uptake and utilisation. This positively affected the development of soybean grains and improved yield. The content of soil macroaggregates increased significantly after reasonable nitrogen addition in anthropogenic soil, the stability was enhanced. And the SOC content in the anthropogenic soil was higher, which positively influence microbial activity and diversity. This microbial activity is directly related to improved crop production, as it aids in the decomposition of organic matter and nutrient cycling [48, 49]. The carbon content of the aggregates at each grain level increased significantly. Soil carbon sequestration capacity improved and the loss of soil nutrients was inhibited. More active organic carbon could be utilised by soybeans, promoting an increase in soybean yield [50]. This finding is consistent with the conclusion that soybean yield is positively correlated with aggregate stability and active carbon fraction content, as shown in Fig. 8. Yang's study also found that the bulk density of the anthropogenic soil decreased significantly, while the maximum water-holding capacity increased. This is important for improving the soil's pore structure and water-holding capacity, thereby promoting plant growth [18]. The addition of nitrogen increases the number of soybean rhizomes, promotes their growth, increases nitrogen-fixing enzyme activity and improves nitrogen-fixing capacity, promoting dry matter accumulation and improving protein content and yield. However, if nitrogen is applied at excessively high concentrations(N4 = 240 kg/ha) during the cultivation of soybeans, it inhibits the growth of root tumours and nitrogen-fixing enzyme activity. This affects the absorption of nutrients by the root system and ultimately leads to a decline in yield [51–53].

5. CONCLUSIONS

This study aims to use HTH-HTC technology to transform weak soil into anthropogenic soil for soybean cultivation, and examining the significant impact of nitrogen on SOC and soybean yield. Compared with weak soils after 112 day’s incubation, the degree of breakdown in the anthropogenic soil aggregates was significantly decreased, the formation pathways were markedly improved, nitrogen addition had a significant effect on the turnover of aggregates. The weakest breakdown of aggregates and the strongest formation occurred when nitrogen was added at a concentration of 120 kg/ha. After soybean cultivation, the stability of aggregates was stronger in the anthropogenic soil compared to the original weak soil, in which MWD = 0.5259 mm, $R_{0.25}$ = 74.72% in A1N3 group, soil aggregates exhibit the highest stability. Compared with weak soil, the SOC of all levels of aggregates increased in the anthropogenic soil. Nitrogen addition promoted the increase of macroaggregate organic carbon content in anthropogenic soils. Different carbon fractions have different evolutionary patterns and characteristics. The carbon content of each component in the anthropogenic soil was higher than that in the weak soil. The highest carbon content of each component was found in aggregates of 0.5-1 mm, 1–2 mm, and > 2 mm in the weak soil, while the carbon content in the anthropogenic soil was mainly concentrated in three grain sizes: 0.25–0.5 mm, 0.5-1 mm, and 1–2 mm. Nitrogen addition significantly increased the carbon content of active carbons, and the carbon content of inert carbons was less affected by the amount of nitrogen added. When nitrogen application concentrations were N2 and N3, microbial communities in the

anthropogenic soil recovered most rapidly, with significant increases in the abundance of the *Bacteroidota*, *Actinomycetot*a and *Basidiomycotas*. Soybean quality and yield were significantly higher in the anthropogenic soil group than in the weak soil group. The highest yield was recorded in group A1N3, with theoretical yield of 3282.88 kg/ha. This was 7.82% higher than the yield in the weak soil group. The study provides a reference and theoretical support for degraded soil remediation and crop yield enhancement.

Declarations

Conflicts of Interest:

The authors declare that there is no conflict of interest regarding the publication of this article.

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Author Contribution

Hui Qiao: Conceptualization, Data curation, Formal analysis, Writing - original draft. Lin Hou: Conceptualization, Visualization, Writing - review & editing. Sikai Huang: Formal analysis, Software, Methodology. Yongping Leng: Investigation, Methodology, Writing - review & editing. Le Zhang: Software, Supervision, Writing - review & editing. Xingyu Lin: Software, Data curation, Validation. Fanyu Meng: Software, Data curation, Writing - review & editing. Yibo Lan: Supervision, Funding acquisition, Project administration, Resources.

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Figures

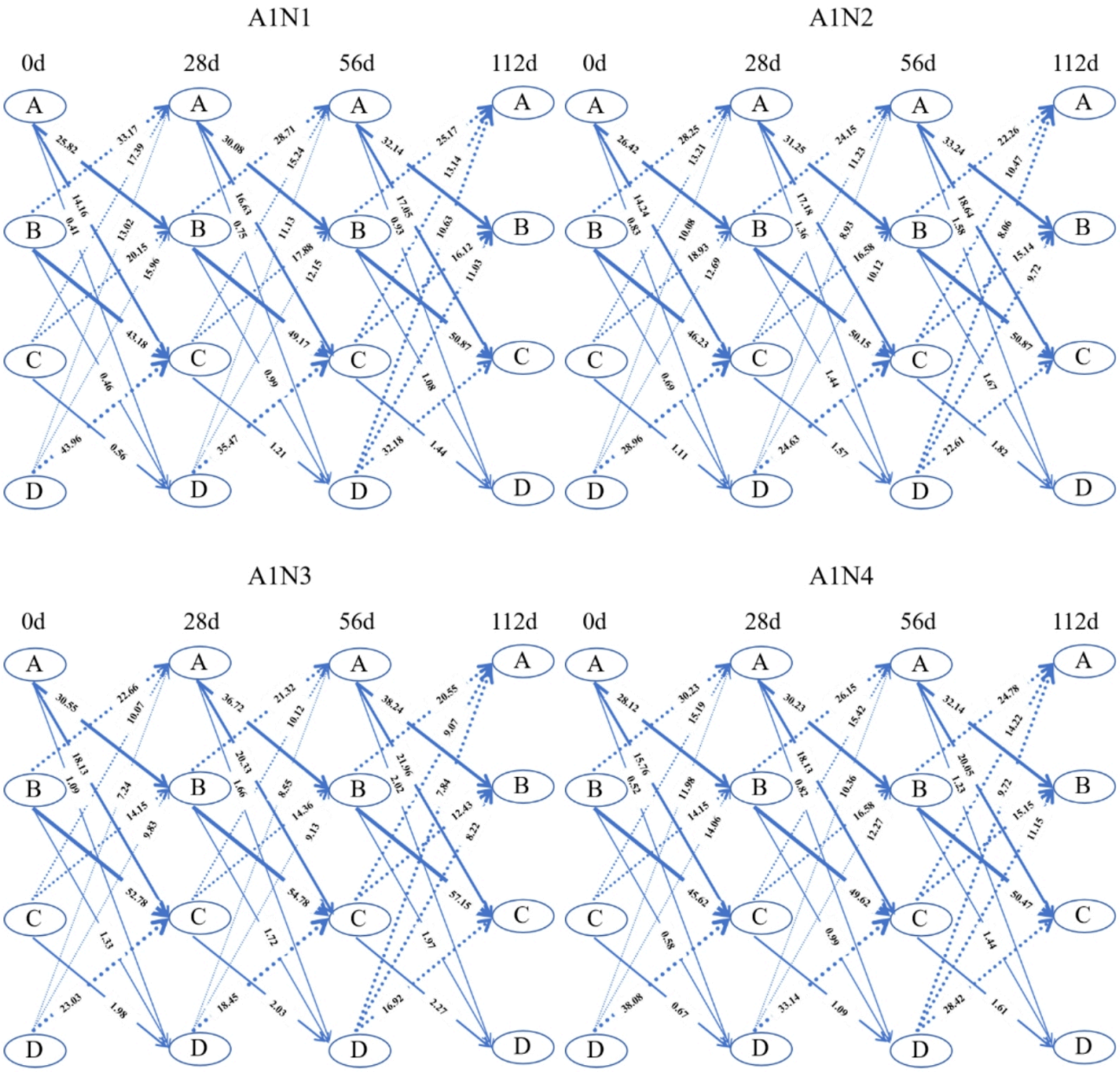


Figure 1

Changes in the transformation paths of anthropogenic soil aggregates.

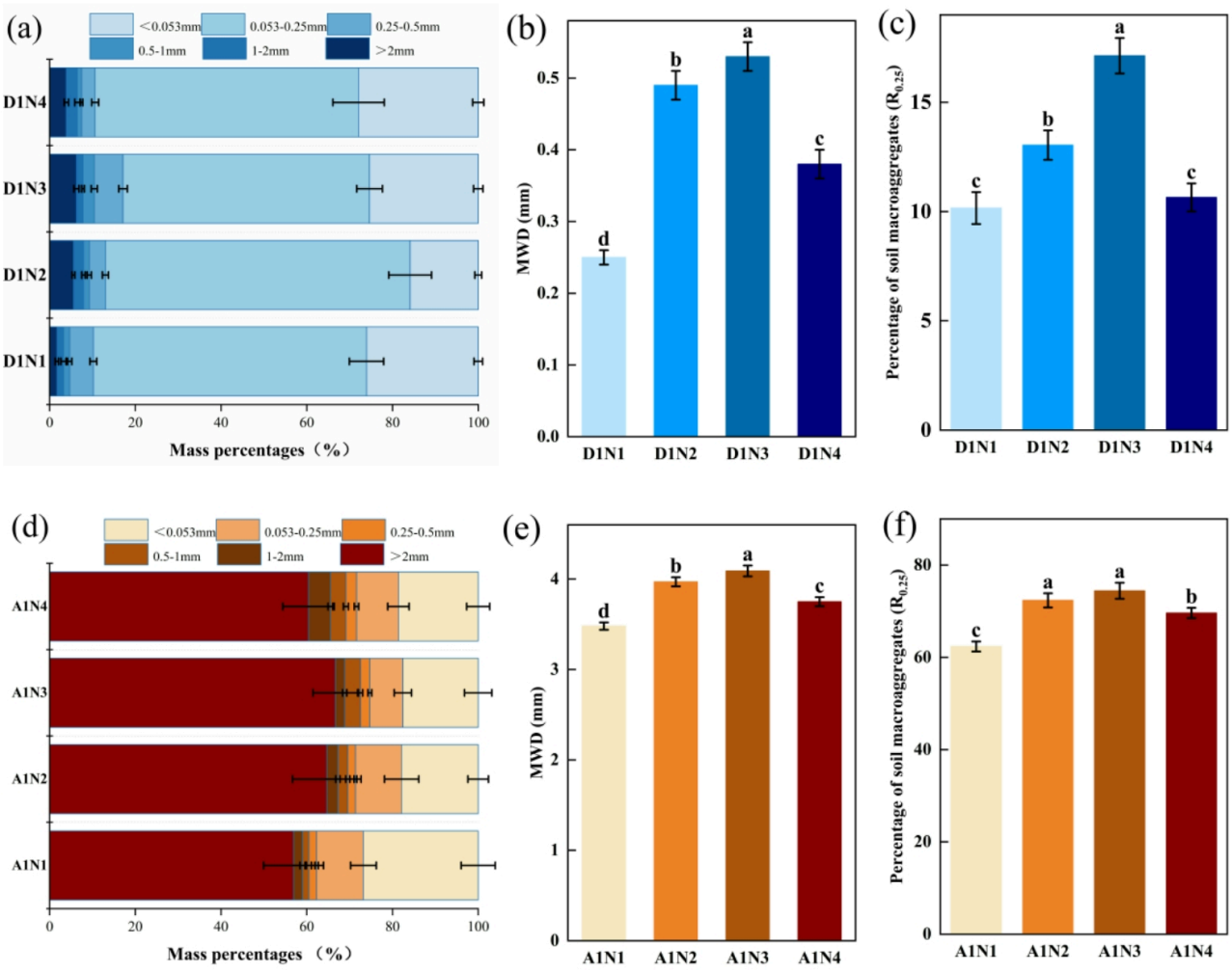


Figure 2

Particle size distribution and stability of soil aggregates.(a): The distribution of aggregates in weak soil, (b): The MWD of weak soil, (c) The $R_{0.25}$ of weak soil, (d): The distribution of aggregates in anthropogenic soil, (e): The MWD of anthropogenic soil, (f): The $R_{0.25}$ of anthropogenic soil.

Note: Different letters in the graph represent significant differences ($p < 0.05$).

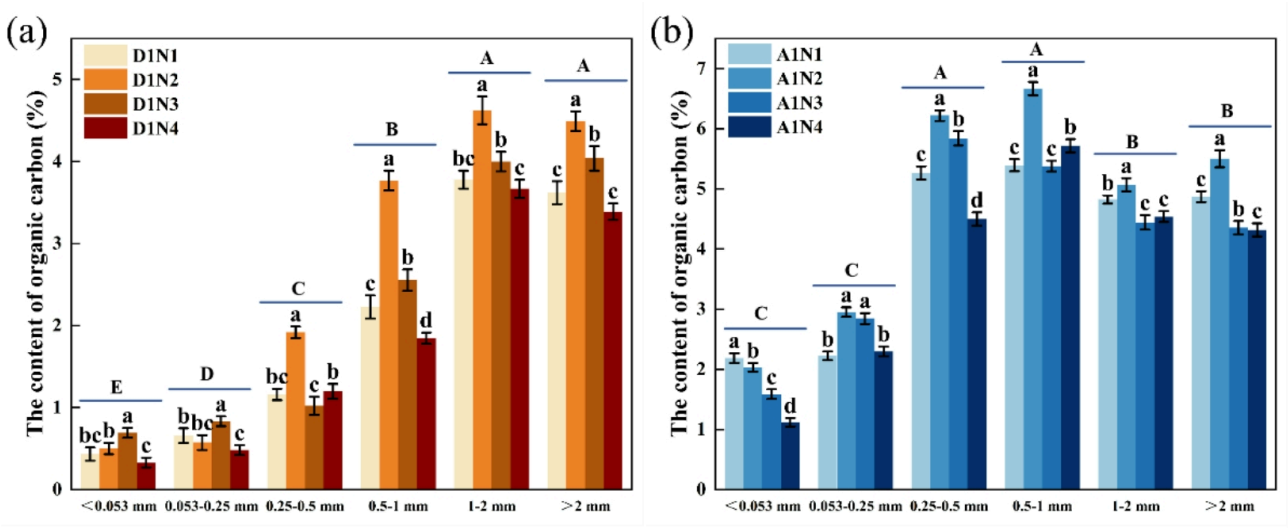


Figure 3

SOC concentrations in soil aggregates. (a): SOC concentrations in weak soil aggregates, (b): SOC concentrations in anthropogenic soil aggregates.

Note: Different letters in the graph represent significant differences ($p < 0.05$).

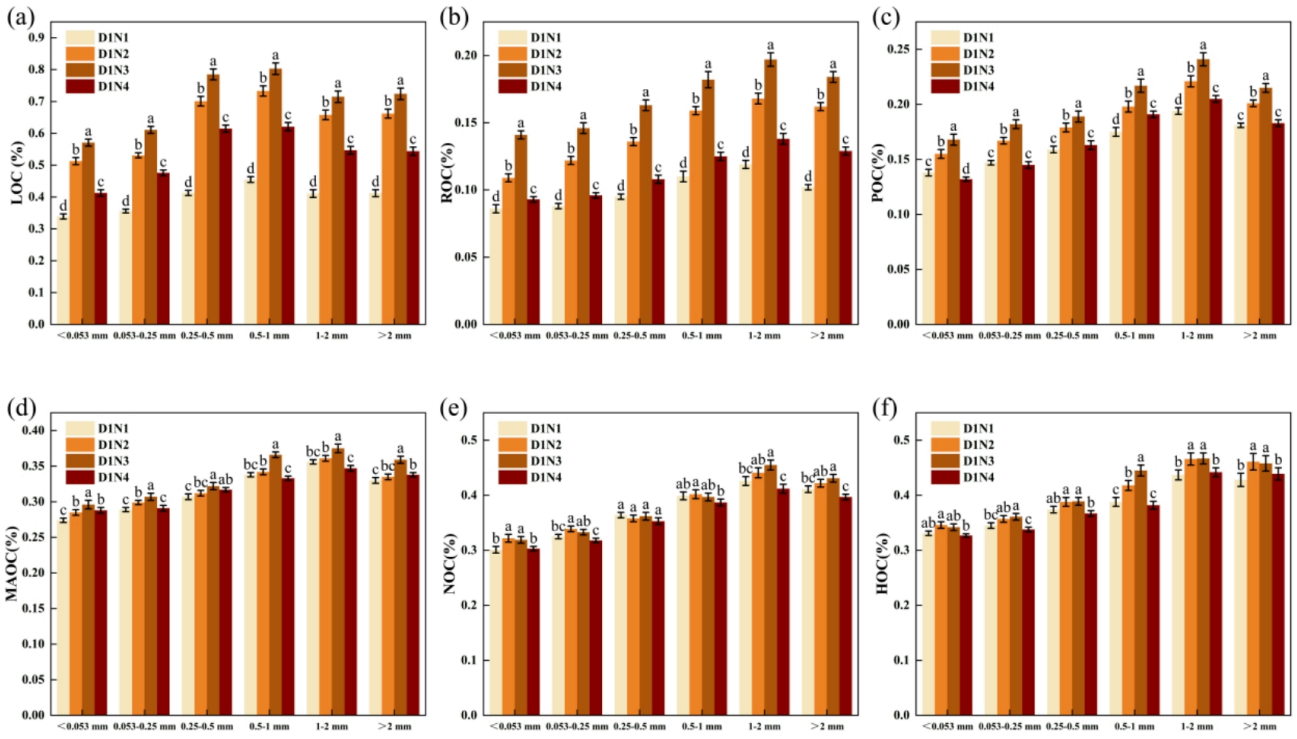


Figure 4

Organic carbon content of fractions in weak soil aggregates. (a): LOC, (b): ROC: (c): POC, (d): MAOC, (e): NOC, (f): HOC.

Note: Different letters in the graph represent significant differences ($p < 0.05$).

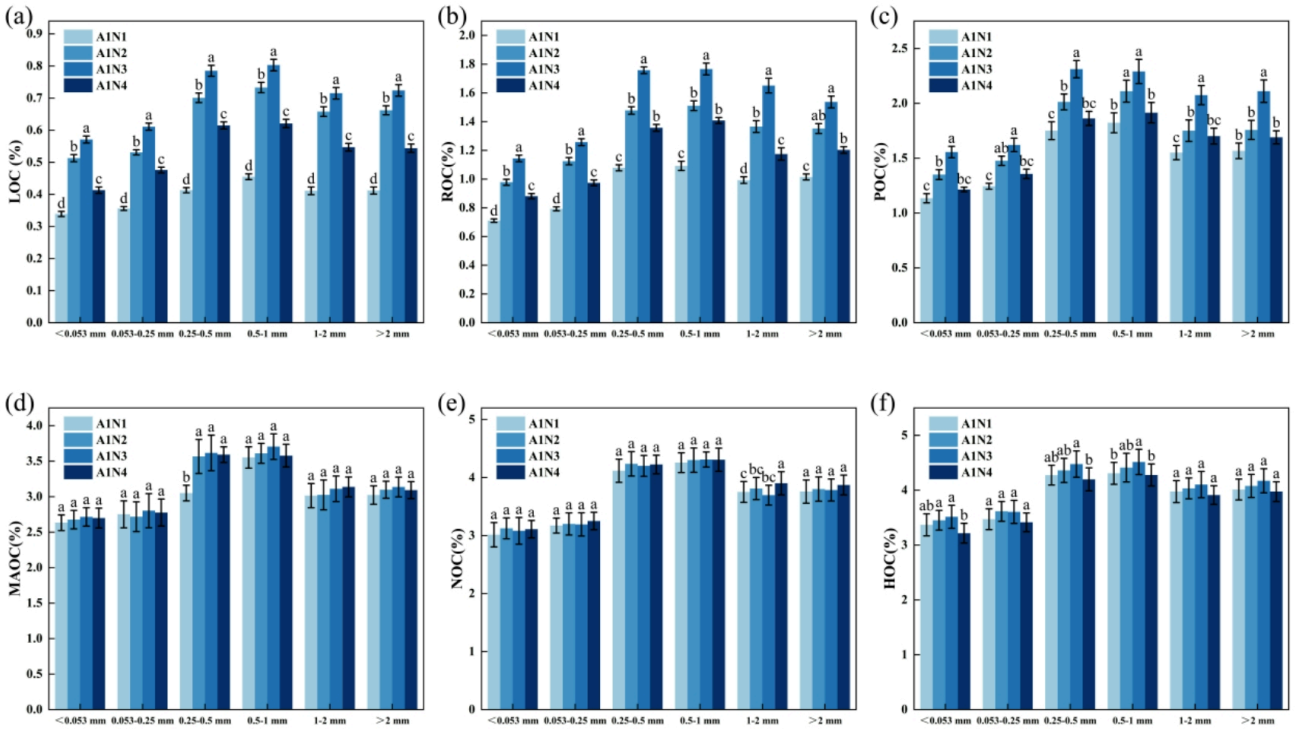


Figure 5

Organic carbon content of fractions in anthropogenic soil aggregates. (a): LOC, (b): ROC: (c): POC, (d): MAOC, (e): NOC, (f): HOC.

Note: Different letters in the graph represent significant differences ($p < 0.05$).

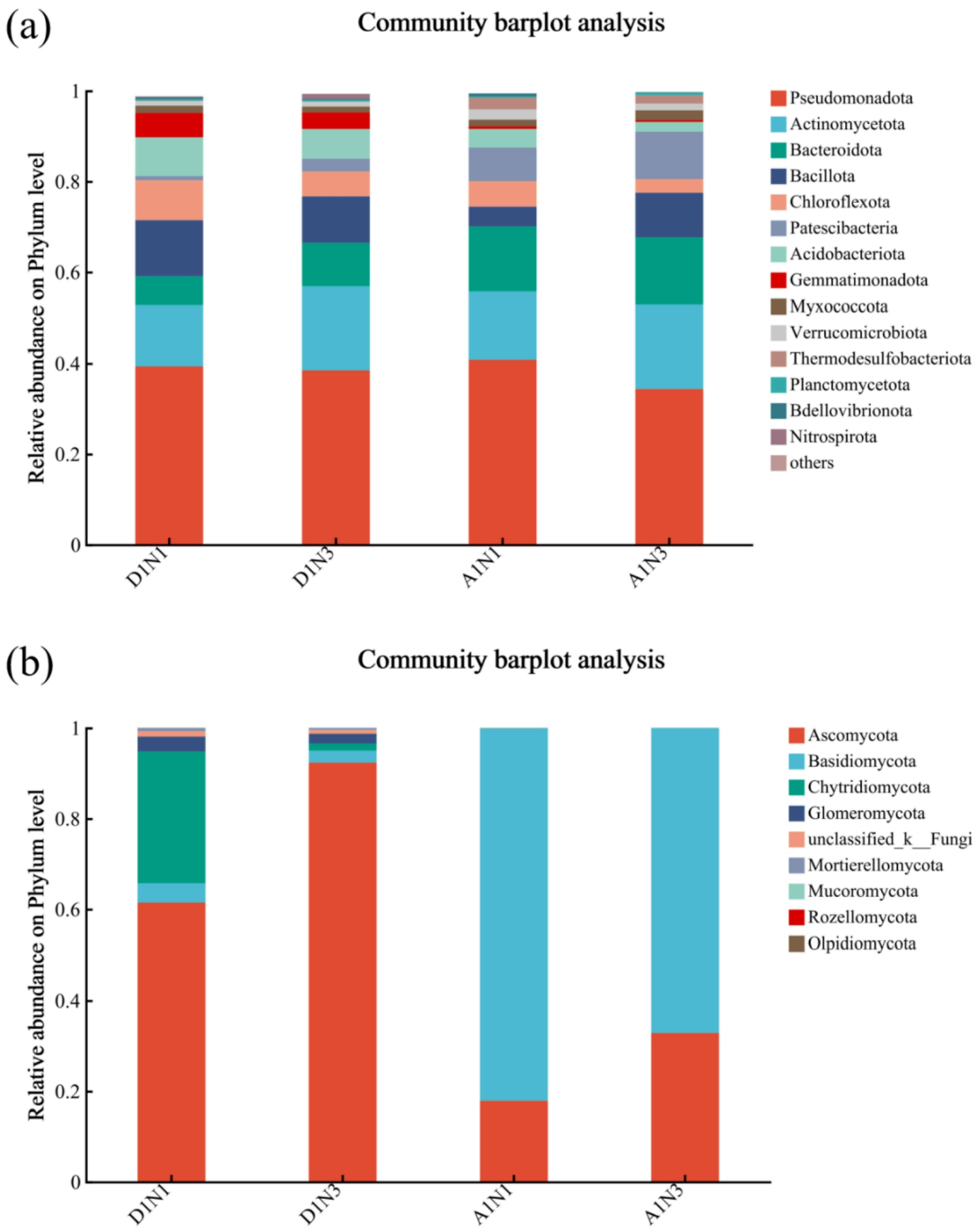


Figure 6

Relative abundance of soil bacterial (a), fungal (b).

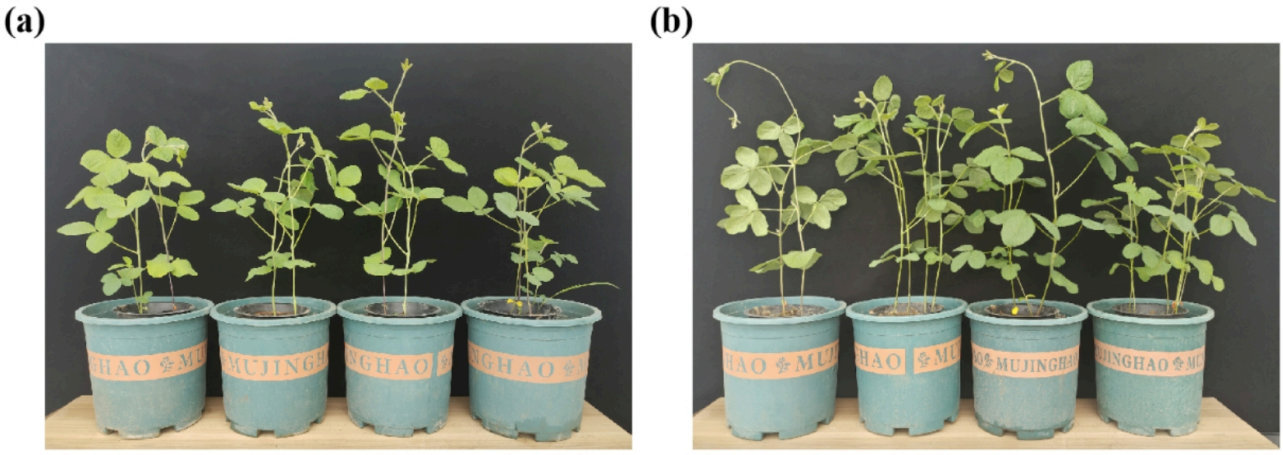


Figure 7

Soybean growth during flowering and pod formation period. (a):D1N1,D1N2,D1N3,D1N4. (b):A1N1,A1N2,A1N3,A1N4.

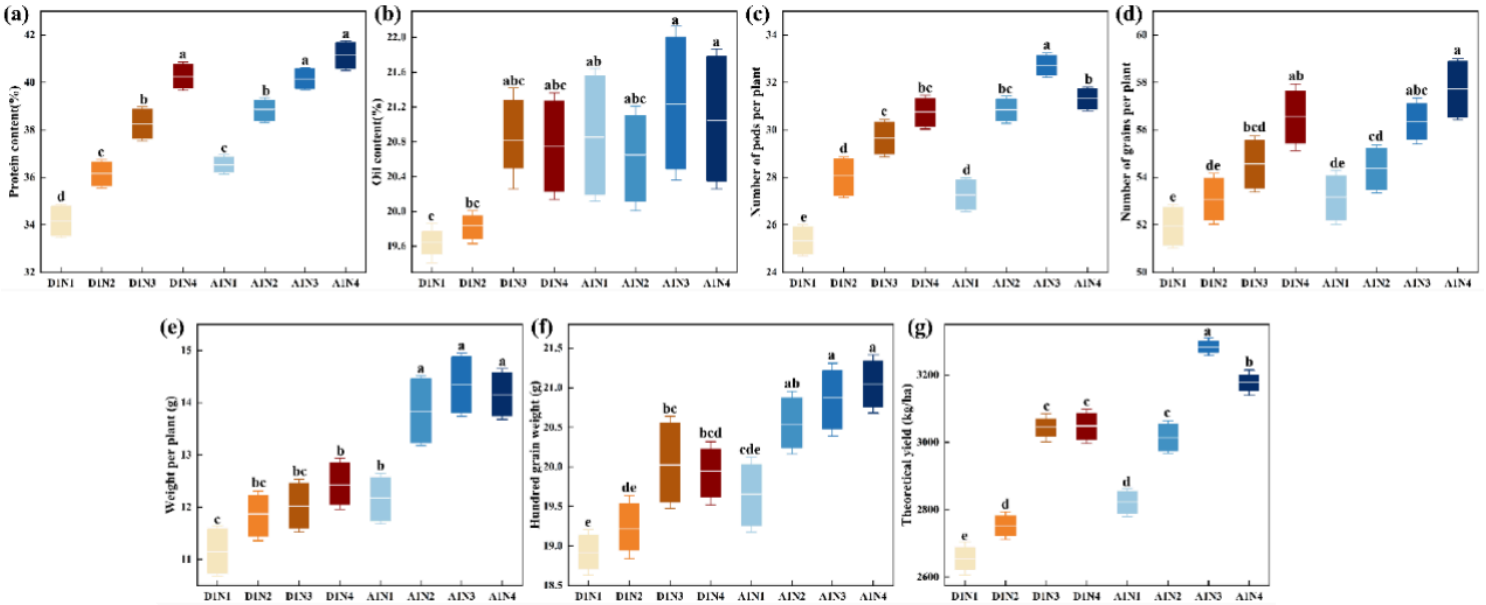


Figure 8

Key indicators and yield of soybean. (a): protein content, (b): oil content, (c):number of pods per plant, (d):number of grains per plant, (e):weight per plant, (f):hundred grains weight, (g): theoretical yield.

Note: Different letters in the graph represent significant differences ($p < 0.05$).

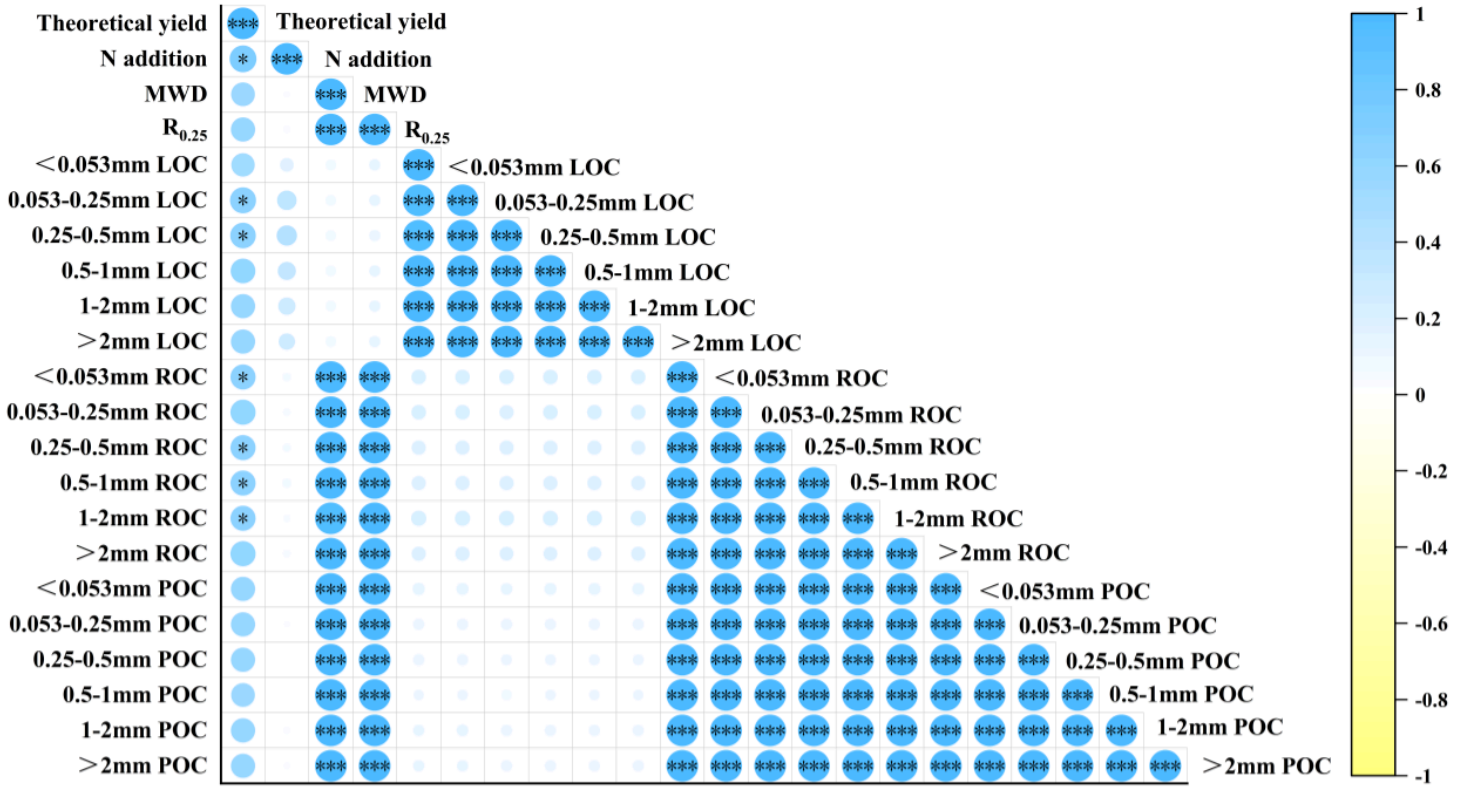


Figure 9

Correlation analysis between key factors and soybean yield (The symbol * represents a significant difference ($0.01 < p < 0.05$), the symbol ** indicates a highly significant difference ($0.001 < p < 0.01$) and the symbol *** indicates the highest significant difference ($p < 0.001$)).

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