ecologies

Article

Linking Rhizosphere Soil Aggregates with Belowground and Aboveground Plant Traits

Md Imam ul Khabir [1], Daphne Topps [1], Jannatul Ferdous Jhumur [1], Anthony Adesemoye [2], Jasmine Brown [1], Antoine Newman [1], Boakai K. Robertson [1], Javed Iqbal [3] and Muhammad Saleem [1,*]

[1] Department of Biological Sciences, Alabama State University, Montgomery, AL 36101, USA
[2] Agrilife Research, Texas A&M University, College Station, TX 77843, USA
[3] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA
* Correspondence: msaleem@alasu.edu

Abstract: Rhizosphere soil ecosystems are represented by the diversity of different soil aggregate-size classes, such as large macroaggregates, small macroaggregates, mesoaggregates, and microaggregates. Though these aggregate-size classes represent distinct biological, chemical, and physical properties, little is known about their dynamics and relationships with belowground and aboveground plant traits. In this study, we examined the relationships of various soil aggregate-size classes and their organic carbon contents with many aboveground and belowground soybean plant traits. Our study revealed several novel and interesting relationships between soil structural properties and plant traits. Notably, small macroaggregates represented a major portion of the rhizosphere soil ecosystem of soybean plants while organic carbon contents decreased with decreasing size of soil aggregates. Only microaggregates showed a significant relationship with root architectural traits, such as length and surface area. Among all soil aggregate size classes, the abundance of small macroaggregates and the organic carbon contents of microaggregates were better correlated with plant traits. In general, organic carbon contents of different soil aggregate-size classes showed positive correlations with leaf trichome density (defense traits) and major macronutrients, such as root P, K, and S contents; while there were mostly negative correlations with some micronutrient (Ca, Mn, Zn, Cu, B, and Mg) contents of roots and shoots. However, the abundance of small macroaggregates mostly positively correlated with the mineral contents of plant roots and shoots. Collectively, the positive and negative correlations of organic carbon contents of different soil aggregate-size classes with trichomes (defense) and physiological traits (micro-mineral contents) suggest their significance in plant nutrition and defense. Though our results suggest the relationships of soil aggregate properties with aboveground and belowground traits, further research is needed to discern the role of soil structural traits in mediating plant growth, development, defense, and physiology.

Keywords: macroaggregates; microaggregates; aboveground and belowground traits; root system architecture; root chemistry; soil organic carbon; plant mineral contents; rhizosphere; root-soil interactions; soil-plant interactions

check for updates

Citation: Khabir, M.I.u.; Topps, D.; Jhumur, J.F.; Adesemoye, A.; Brown, J.; Newman, A.; Robertson, B.K.; Iqbal, J.; Saleem, M. Linking Rhizosphere Soil Aggregates with Belowground and Aboveground Plant Traits. *Ecologies* **2023**, *4*, 74–87. https://doi.org/10.3390/ecologies4010007

Academic Editor: José Ramón Arévalo Sierra

Received: 17 January 2023
Revised: 28 January 2023
Accepted: 30 January 2023
Published: 8 February 2023

1. Introduction

The rhizosphere soil is a complex environment that includes a diversity of soil aggregates, both in terms of their sizes and shapes. The physical component of soil is characterized by different aggregate-size classes, ranging from micro- to macro-aggregates. Classically, soil aggregates are grouped into macroaggregates (>0.25 mm) and microaggregates (<0.25 mm) [1,2]. However, some studies have further classified and categorized them into four aggregate-size classes, such as large macroaggregates (>2000 µm), small macroaggregates (<2000–500 µm) [3], mesoaggregates (<500–250 µm) [1,4], and microaggregates (<250 µm) [5]. Though different soil aggregate-size classes differ in their physical, chemical, and biological properties, both macroaggregates and microaggregates can have several distinct differences. These include but are not limited to: (i) carbon turnover increases with decreasing soil aggregate size; (ii) macroaggregates generally possess more organic carbon than microaggregates; (iii) the size of soil aggregates is inversely proportional to the amount of energy required to break them; and (iv) the decomposition generally increases with decreasing soil aggregate sizes [2,6,7]. Nevertheless, soil aggregates are considered important indicators of essential soil properties that determine soil nutrient retention, cycling, porosity, and erosion resistance [8]. Aggregation of soil particles into different soil aggregate-size classes increases soil capacity to encapsulate soil organic carbon (SOC) by improving the physical protection of organics to reduce the biodegradation mediated by the soil microbial activities [9].

Different agronomic practices can significantly alter the composition and relative abundance of various aggregate-size classes in agricultural soils. For instance, conventional soil tillage practices, such as intensive plowing, can potentially break the soil macroaggregates into smaller, finer and/or microaggregates [10–12]. Contrarily, the amendment of soil with organic materials or conservation tillage can potentially increase the proportion of soil macroaggregates. Several studies have reported a positive correlation between soil organic carbon and the formation of macroaggregates in soils under different farm management practices [2,7,12]. Furthermore, the alternation between wet and dry conditions can potentially break macro-aggregates into other aggregate-size classes, thus altering the soil structure [13–15]. Recently, our results suggested that cover crop increased and decreased the proportion of soil macroaggregates and microaggregates, respectively [1]. There has been a great deal of work on the impact of soil texture, aggregate stability, and other edaphic properties on root system architecture and growth parameters of crop plants [16,17]. Similarly, several studies have tested the impact of soil amendments on soil biophysical conditions [18,19]. Although the biochemical and physical properties of soil aggregates are described in several studies, little is known about their relationship with plant traits under natural settings.

Few studies have tried to link the relationship between soil aggregate types and plant traits. For instance, Kohler et al. (2010) studied the relationship between soil aggregates and root biomass of *Lactuca sativa* under microbial influence [20]. In another study, root biomass was negatively correlated with various soil aggregate classes [21]. Nevertheless, some studies have tested the impact of individual aggregates on various plant growth parameters, such as germination and root growth [22]. Though not empirically tested, it is commonly perceived that the nature and type of soil aggregates may influence plant growth and development. For instance, macro- than micro-aggregates may have relatively more organic matter and nutrient levels and, consequently, these properties may influence plant growth via better nutrient availability, mobility, and aeration [23–25]. Nevertheless, we know very little about the mutual relationships between soil aggregates and plant traits (belowground and aboveground).

In this study, using soil and plant samples from soybean crop grown under different conditions (seed treatments), we investigated the correlation of soil aggregate-size classes with several aboveground and belowground traits of soybean plants. This study aimed to explore the relationship of essential soil properties, such as different aggregate-size classes and their properties (abundance and organic carbon contents), with shoot and root physical and chemical traits. We hypothesized that both relative abundance (physical property) and organic carbon contents (chemical property) of different soil aggregate-size classes would demonstrate correlation with various belowground (root) and aboveground (shoot) traits of soybean plants, though nature of these correlations will depend on soil aggregate properties and types of plant traits. According to our knowledge, this is the first study that has attempted to explore the links between soil structural properties; for instance, the properties of soil aggregate-size classes with several plant physiological, mineral, and defense traits.

2. Materials and Methods

2.1. Belowground and Aboveground Plant Data Collection

We collected soil and plant samples from the soybean experimental plots that were established at the Henry J. Stumpf International Wheat Center (Grant, NE, USA). The experimental soil had the following properties at the time of sowing: The soil contained 47% sand, 42% silt, and 11% clay. It had a pH of 6.8, with other nutrients at 0.58 mg kg^{-1} soluble salt, 2.2% organic matter, 9.3 mg kg^{-1} of nitrate nitrogen (NO_3-N), 6 mg kg^{-1} of phosphorus (P_2O_5), and 302 mg kg^{-1} of potassium (K). This experiment was conducted in June 2018, while rhizosphere soil and plant samples were collected at the soybean reproductive stage six (R6) in August 2018. We took soil and plant samples from soybean plants that were grown under different conditions, such as seed treatments. Since the major objective of this study was to determine the relationships of soil aggregate properties (i.e., abundance and organic carbon contents) with plant traits on average, testing similar relationships at each seed treatment level was neither possible (due to low replicates or sample numbers) nor our objective. The soil and plant samples were collected and processed as described recently [26]. The samples were taken at the reproductive maturity of the soybean crop. The details about experimental set up, inoculation, sampling procedure, collection, and processing of belowground and aboveground plant traits were recently reported [26] and also given (Supplementary Materials). The root architectural traits were profiled using Winrhizo, whereas the leaf trichomes were counted under the microscope. Both root and shoot tissues were analyzed for mineral contents as reported recently [26,27]. Unless otherwise stated, the plant root and shoot mineral contents (P, K, Ca, Mg, S) are expressed as the percentage (%) of the dry matter, while the contents of other minerals (Zn, Fe, Mn, Cu, Mo, and B) are expressed as ppm (mg/Kg).

2.2. Profiling of Different Soil Aggregate-Size Classes

At reproductive maturity, the rhizosphere soil samples (~500 g) were collected into the plastic bags from the above-mentioned soybean field treatments. These soil samples were gently crumbled to break the large soil clods along the planes of weakness before drying and sieving [1,28]. The soil samples were sieved into four aggregate-size classes that included large macroaggregates (particle size >2000 µm), small macroaggregates (<2000–500 µm), mesoaggregates (<500–250 µm), and microaggregates (<250 µm). Given discrepancies in terms of the advantages and disadvantages of using different soil sieving approaches, we selected dry rather than wet sieving for profiling the soil aggregate-size classes [29]. Briefly, we choose dry sieving because it causes relatively less damage to the soil structure and shows the biologically active carbon pools in the rhizosphere soil ecosystems [1]. The aggregate-size classes profiled through this approach are often used to determine soil resistance against different environmental stresses, such as erosion and porosity [30]. After sieving, we calculated the relative abundance (% of total) of different soil aggregate-size classes in each sample. The relative abundance (% proportion) of each soil aggregate-size class was determined by dividing its mass by the total mass of all soil aggregates in the same sample, followed by multiplying by 100.

2.3. Analysis of Organic Carbon Contents of Different Soil Aggregate-Size Classes

The plant roots and debris were removed using forceps. All sieved aggregate-size classes were kept in 50 mL falcon tubes at the room temperature till their analysis for soil organic carbon contents. Before analysis, the samples belonging to different soil aggregate-size classes were treated with sulfurous acid (H_2SO_3) to remove different inorganic forms of the carbonates (e.g., inorganic carbon). Next, the organic carbon contents were determined using the resistance furnace method, as described previously [20]. Briefly, samples of different soil aggregate-size classes were ignited in the oxygen-rich combustion chamber at 1350 °C. After that, the aliquots of the combustion gas were passed through an infrared absorption detector to quantify the organic carbon contents [4,21,22].

2.4. Data Analysis

In this study, as described elsewhere, we investigated crop plant traits in five different treatments that had at least four experimental replicates. Considering the significance of plant traits and soil structure, we used this opportunity to discern the relationship between plant traits and soil aggregate-size classes. In all the analyses, we performed linear regression followed by one-way analysis of variance (ANOVA) to test the relationship of soil aggregate-size classes with aboveground and belowground plant traits, as outlined in the result section (Figures 1c–6, Tables 1–4). To determine differences in the relative proportion (% of total) of different aggregate-size classes in the soybean rhizosphere soil, we conducted ANOVA, followed by the Fisher's test at a significance of 0.05 (Figure 1b). Overall, these are typical tests for exploring correlations between variables in experimental studies.

3. Results

3.1. Composition and Properties of Soil Aggregate Size-Classes

After separating soil aggregate-size classes, we found that small-macroaggregates made up the dominant portion of the total soil mass, followed by mesoaggregates, large-macro macroaggregates and microaggregates (Figure 1a). Regarding their properties, we found a gradual decline in the organic carbon contents with decreasing size of soil aggregates (Figure 1b). Moreover, a positive correlation between organic carbon contents of soil aggregate-size classes was observed, though it was absent between macroaggregates and microaggregates (Figure 1c).

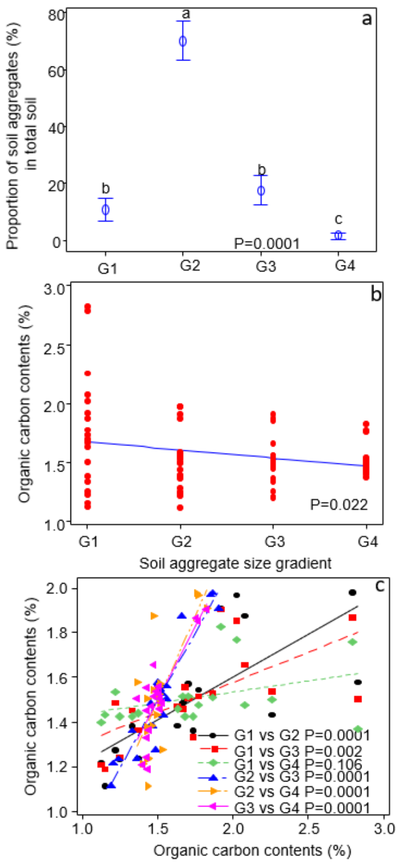

Figure 1. (**a**) The relative proportion of different soil aggregate-size classes, such as large macroaggregates (G1), small macroaggregates (G2), mesoaggregates (G3), and microaggregates (G4). Lack of letter sharing on bars represents significant statistical difference. (**b**) The soil organic carbon contents across the soil aggregate-size gradient from large macroaggregates to microaggregates as determined by the linear regression. (**c**) The correlation between organic carbon contents of various soil aggregate-size classes as determined by the linear regression.

3.2. Relationship of Soil Aggregate-Size Classes with Plant Growth Parameters

We also investigated the relationship between different plant growth parameters and various soil aggregate size classes, and only microaggregates positively correlated with root length and surface area (Figure 2).

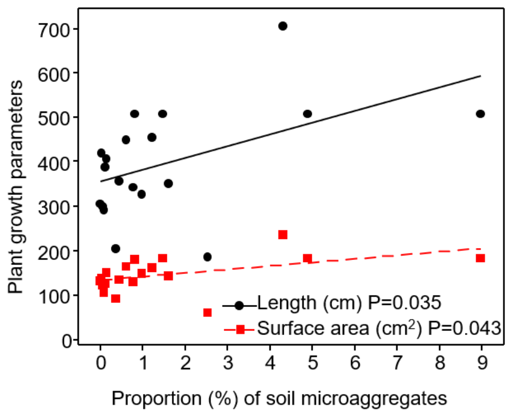

Figure 2. The relationship between the abundance (proportion of total) of soil microaggregates and root length (cm) and area (cm^2), as determined by the linear regression. Since both root length and area had different units, and are also plotted together, we named the *y*-axis "plant growth parameters to represent root traits simultaneously".

3.3. Relationship of Large-Macroaggregates (>2000 µm) with Aboveground and Belowground Traits

The relative abundance of large macroaggregates in soil showed a positive relationship with leaf traits, such as adaxial (up) and abaxial (down) trichome density. Moreover, the large macroaggregates in soil also positively correlated with the P and K contents of fine roots (FR) and large root (LR) K contents. Other than that, the large macroaggregates showed a negative correlation with the Ca, Zn, Fe, Mn, and Cu contents of FR, while the same relationship was observed for the K, Ca, Mn, and Cu contents of LR (LR), on average, across all samples (Table 1). Moreover, we also investigated the relationship of soil large macroaggregates with plant traits. Both the S and B contents of LR correlated positively; however, the Mo contents of LR correlated negatively with the abundance of large macroaggregates (Figure 3).

Table 1. Relationship between abundance of large macroaggregates (>2000 µm) and plant traits.

Large Aggregate (>2000 µm)	*Linear Regression Coefficient (R^2)*	*Probability Value (P)*	*Relationship*
Shoot traits			
Up trichome	$R^2 = 0.34$	$p = 0.006$	+
Down trichome	$R^2 = 0.25$	$p = 0.023$	+
Fine root traits			
Fine root P contents (%)	$R^2 = 0.18$	$p = 0.056$	+
Fine root K contents (%)	$R^2 = 0.26$	$p = 0.02$	+
Fine root Ca contents (%)	$R^2 = 0.33$	$p = 0.0008$	-
Fine root Zn contents (%)	$R^2 = 0.38$	$p = 0.004$	-
Fine root Fe contents (%)	$R^2 = 0.30$	$p = 0.012$	-
Fine root Mn contents (%)	$R^2 = 0.32$	$p = 0.008$	-
Fine root Cu contents (%)	$R^2 = 0.27$	$p = 0.017$	-
Large root traits			
Large root K contents (%)	$R^2 = 0.38$	$p = 0.004$	+
Large root Ca contents (%)	$R^2 = 0.21$	$p = 0.038$	-
Large root Mn contents (%)	$R^2 = 0.27$	$p = 0.019$	-
Large root Cu contents (%)	$R^2 = 0.18$	$p = 0.059$	-

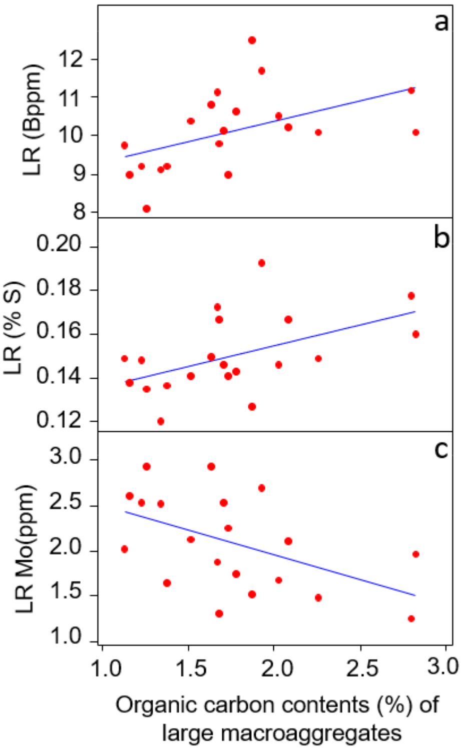

Figure 3. The relationship between the organic carbon contents (%OC) of soil large macroaggregates with the B (**a**), S (**b**), and Mo (**c**) contents of LR, as determined by the linear regression. Only significant ($p < 0.05$) relationships are plotted.

3.4. Relationship of Small Macroaggregates (<2000–500 μm) with Plants Traits

Likewise using linear regression, we tested the relationship of small macroaggregates with various plant traits (Table 2). The relative abundance of small macroaggregates in soil showed a negative relationship with leaf traits, such as adaxial and abaxial trichome density. In addition, their abundance in the soil positively correlated with shoot S and Mn contents. These aggregates showed a positive correlation with the Ca, Zn, Fe, Mn, and Cu contents of FR, and the Cu contents of the LR. However, the relative abundance of small macroaggregates in soil negatively correlated with the P and K contents of FR, and the K and B contents of LR (Table 2). The organic carbon contents of small macroaggregates correlated positively with the B, K, and S contents of LR (LR). Similarly, these correlated positively with the Mg, P, and K contents, and negatively with Zn, Ca, and B contents of FR (Figure 4). The leaf abaxial trichomes positively correlated with the organic carbon contents of small-macroaggregates in the rhizosphere soil. However, the shoot contents of Ca, Mn, and S negatively correlated with the organic carbon contents of small-macroaggregates (Figure 4).

3.5. Relationship of Mesoaggregates (<500–250 μm) to Plant Traits

Moreover, using linear regression, we also tested the relationship between mesoaggregates and various plant traits (Table 3). Interestingly, the relative abundance of large macro-aggregates in soil (% of total) showed a positive relationship with the P and B contents of LR and the P contents of FR. However, the relative abundance of mesoaggregates negatively correlated with the Mo and Mn contents of LR and plant shoots (Table 3). The organic carbon contents of mesoaggregates positively correlated with the S, B, and K contents of LR. Similarly, both adaxial and abaxial trichome density positively correlated with the organic carbon contents of mesoaggregates. The organic carbon contents of mesoaggregates positively correlated with the P and K contents, while negatively correlated with the B, Zn, and Cu contents of LR. The shoot B, S, and Mn contents positively correlated with the organic carbon contents of mesoaggregates (Figure 5).

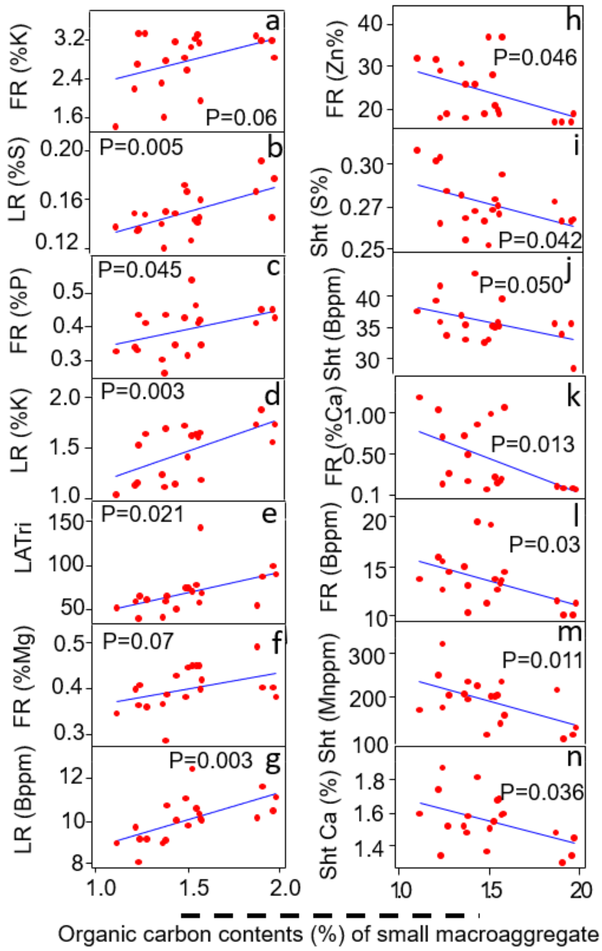

Figure 4. The relationship of organic carbon contents (%OC) of soil small macroaggregates with plant traits as determined by the linear regression (**a**–**n**). Only significant (p < 0.05) relationships are plotted. The abbreviations are LR (large roots), FR (fine roots), Sht (shoot), LAdTri (leaf adaxial trichome density), and LAbTri (leaf abaxial trichome density). The trichome density was number of trichomes per 0.29 cm^2 of soybean leaf area.

Table 2. Relationship between abundance of small macroaggregates (<2000–500 µm) and plant traits.

Small Macro-Aggregates (<2000–500 µm)	*Linear Regression Coefficient (R^2)*	*Probability Value (P)*	*Relationship*
Plant traits			
Up trichome	$R^2 = 0.28$	$p = 0.024$	-
Down trichome	$R^2 = 0.24$	$p = 0.023$	-
Shoot traits			
Shoot S content (%)	$R^2 = 0.21$	$p = 0.043$	+
Shoot Mn content (%)	$R^2 = 0.46$	$p = 0.017$	+
Fine root traits			
Fine root P contents (%)	$R^2 = 0.40$	$p = 0.003$	-
Fine root K contents (%)	$R^2 = 0.25$	$p = 0.029$	-
Fine root Ca contents (%)	$R^2 = 0.33$	$p = 0.008$	+
Fine root Zn contents (%)	$R^2 = 0.22$	$p = 0.035$	+
Fine root Fe contents (%)	$R^2 = 0.23$	$p = 0.034$	+
Fine root Mn contents (%)	$R^2 = 0.22$	$p = 0.035$	+
Fine root Cu contents (%)	$R^2 = 0.30$	$p = 0.012$	+
Large root traits			
Large root K contents (%)	$R^2 = 0.41$	$p = 0.002$	-
Large root Cu (ppm)	$R^2 = 0.20$	$p = 0.047$	+
Large root B content (%)	$R^2 = 0.56$	$p = 0.0001$	-

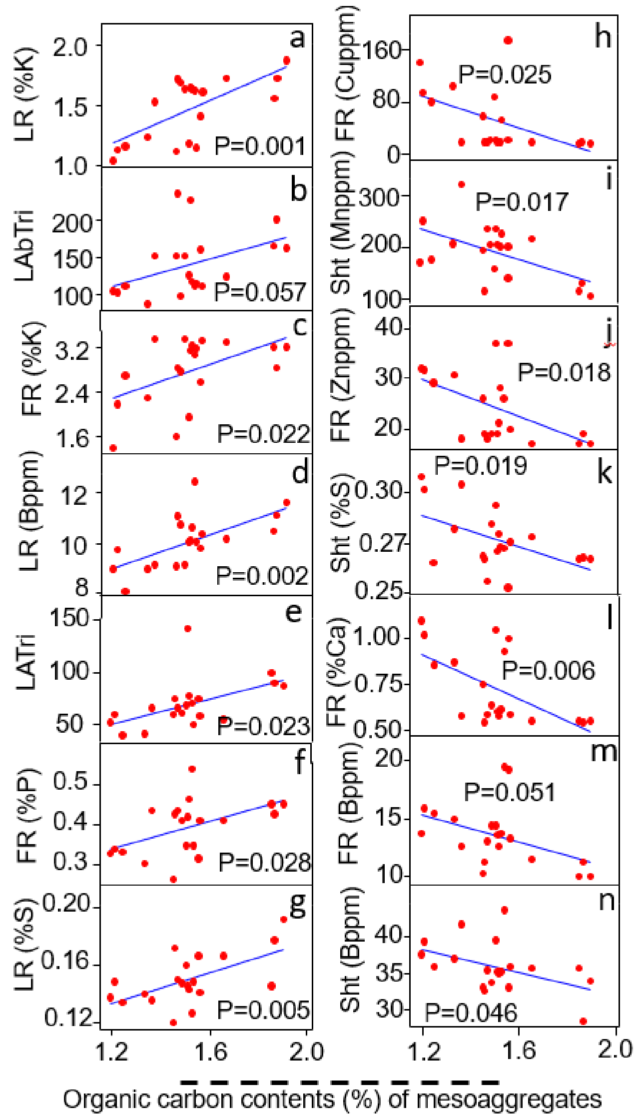

Figure 5. The relationship between the organic carbon contents (%OC) of soil mesoaggregates and plant traits, as determined by linear regression. Only significant ($p < 0.05$) relationships are plotted (**a**–**n**). The abbreviations are LR (large roots), FR (fine roots), Sht (shoot), LAdTri (leaf adaxial trichome density), and LAbTri (leaf abaxial trichome density). The trichome density was number of trichomes per 0.29 cm^2 of soybean leaf area.

Table 3. Relationship between abundance of mesoaggregates (<500–250 µm) and plant traits.

Meso-Aggregates (<500–250 µm)	*Linear Regression Coefficient (R^2)*	*Probability Value (P)*	*Relationship*
Shoot traits			
Shoot Mn content (ppm)	$R^2 = 0.18$	$p = 0.08$	-
Fine root traits			
Fine root P content (%)	$R^2 = 0.19$	$p = 0.07$	+
Large root traits			
Large root P content (%)	$R^2 = 0.16$	$p = 0.087$	+
Large root B content (%)	$R^2 = 0.47$	$p = 0.0001$	+
Large root Mo content (ppm)	$R^2 = 0.45$	$p = 0.0001$	-

3.6. Relationship of Microaggregates (<250 µm) to Plant Traits

The relative abundance of microaggregates also showed positive correlation with the K and B contents of LR, and the Zn contents of the FR. However, the relative abundance of these aggregates showed a negative correlation with the Ca, Fe, Mn, and Cu contents of LR, and the B contents of shoot tissues (Table 4). The organic carbon contents of microaggregates positively correlated with the S, B, and K contents of LR in the rhizosphere soil. Similarly, the organic carbon contents of microaggregates positively correlated with the P and K contents, while negatively correlating with the B, Zn, and Cu contents of LR. Similar to mesoaggregates, both adaxial and abaxial trichome density positively correlated with the organic carbon contents of microaggregates. Finally, the B, S, and Mn contents of the shoots negatively correlated with the organic carbon contents of microaggregates (Figure 6).

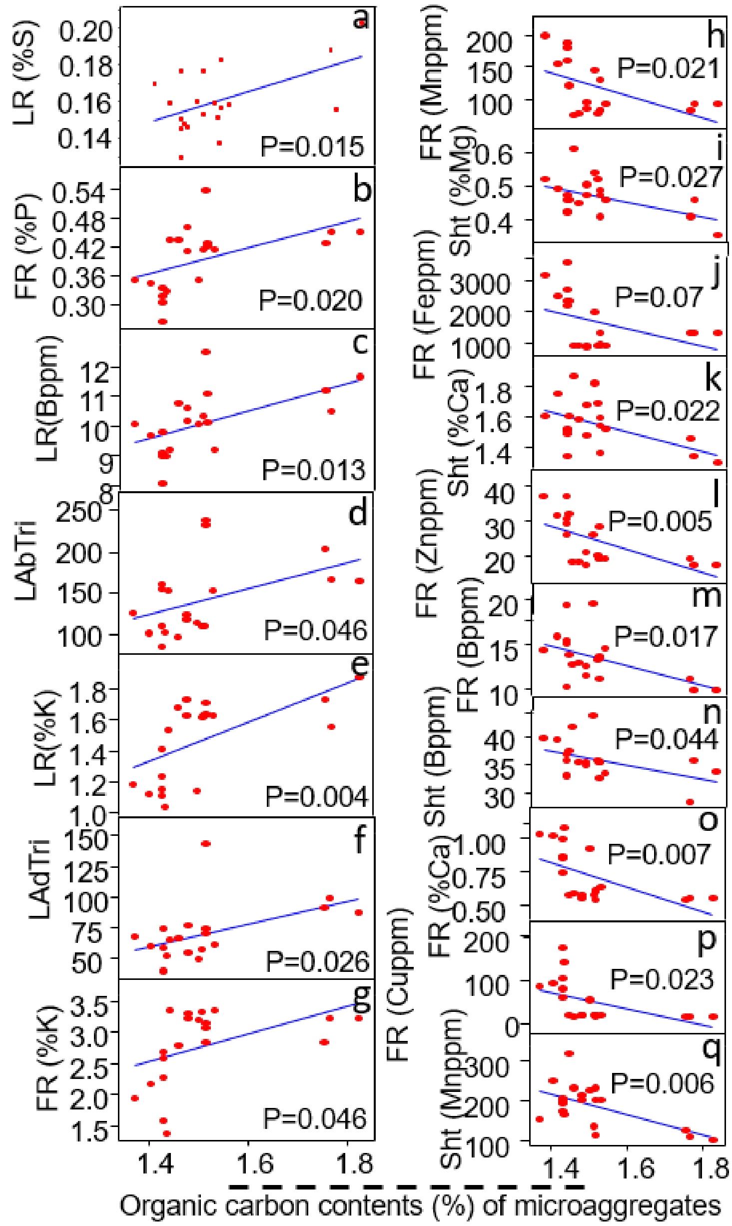

Figure 6. The relationship of organic carbon contents (%OC) of soil microaggregates to plant traits as determined by linear regression. The abbreviations are LR (large roots), FR (fine roots), Sht (shoot), LAdTri (leaf adaxial trichome density), and LAbTri (leaf abaxial trichome density). The trichome density was number of trichomes per 0.29 cm^2 of soybean leaf area. Only significant ($p < 0.05$) relationships are plotted (**a**–**q**).

Table 4. Relationship between abundance of microaggregates (<250 μm) and plant traits.

Micro-Aggregates (<500–250 μm)	*Linear Regression Coefficient (R^2)*	*Probability Value (P)*	*Relationship*
Shoot traits			
Shoot Zn content (%)	R^2 = 0.39	p = 0.036	-
Shoot B content (%)	R^2 = 0.36	p = 0.038	-
Fine root traits			
Fine root Ca content (%)	R^2 = 0.35	p = 0.034	-
Fine root Zn content (%)	R^2 = 0.28	p = 0.067	+
Fine root Fe content (%)	R^2 = 0.33	p = 0.09	-
Fine root Mn content (%)	R^2 = 0.33	p = 0.037	-
Fine root Cu content (%)	R^2 = 0.34	p = 0.058	-
Large root traits			
Large root K content (%)	R^2 = 0.35	p = 0.031	+
Large root Cu content (%)	R^2 = 0.30	p = 0.053	-
Large root B content (%)	R^2 = 0.41	p = 0.024	+

4. Discussion

Soil structure plays a significant role in soil quality, health, and functioning [31]; while soil aggregation is considered an important indicator of soil structure [1]. The aggregation of mineral particles into various aggregate-size classes (from microaggregates to macroaggregates) determine the quality and level of soil aggregation. Thus, soil aggregate-size classes are the fundamental units of soil systems, while their physical stability is an important indicator of essential soil properties [32]. Soils with different soil aggregate-size classes could potentially enhance the capacity of soils to sequester carbon, moisture, and other essential nutrients [33]. Therefore, soil aggregates might have important role in plant growth, development, and defense, though this has not been empirically tested. In this study, we investigated the relationship between soil aggregate abundance and organic carbon contents with belowground and aboveground plant traits.

Large macroaggregates made up a relatively significant fraction of the soil environment; while these closely related to important aboveground defense traits—such as leaf trichome density—and nutritional traits—such as the content of major plant macronutrients, including the P and K contents of LR and the K contents of FR. Interestingly, the organic carbon contents of large macroaggregates were also shown to be relatively higher and positively correlated with other essential nutrients, such as the S and B contents of LR. However, interestingly, the minerals content of several micronutrients—such as Ca, Zn, Fe, Mn, and the Cu of LR, as well as the K, Ca, Mn, and Cu contents of LR—correlated negatively with the abundance of large macroaggregates (Table 1, Figure 3). The positive correlation of plant mineral contents with the properties of large macroaggregates could be due to their better organic carbon contents (Figure 1b), which are considered supportive of soil fertility [34]; whereas the negative correlations with several micronutrient contents could be due to their poor interaction with plant roots and/or sequestration of these nutrients by organic carbon complexes [35]. In addition to their abundance and organic carbon contents, other properties of large macroaggregates might also determine plant tissue nutrient contents; for instance, nutrient contents, resident microbial taxa, orientation and exposure of roots, and C: N ratio—including other edaphic properties and plant factors, such as the properties of root architectural traits [36,37]. However, further research is required to estimate the relative influence of soil and plant factors in mediating the impacts of large macroaggregates on plant nutrition.

Interestingly, small-macroaggregates made up the largest proportion of the soybean rhizosphere soil environment (Figure 1a). Previously, some studies reported the impact of crop types and diversity on composition and relative abundance of different soil aggregate-size classes [1], though there was a greater abundance of macroaggregates in soybean rhizosphere soil under different management practices, such as no tillage, applications of organic manures, and biofertilizers [38–40]. Unlike large ones, the abundance of small-macroaggregates negatively correlated with leaf traits, such as trichome density (adaxial and abaxial); though their carbon contents positively correlated with these traits. These results imply the significance of organic carbon contents for mediating plant defense traits, such as trichomes [41]. In correspondence with their greater abundance in the rhizosphere soil, the small-macroaggregates correlated positively with most aboveground and belowground plant traits, such as shoot S and Mn contents, and the Ca, Zn, Fe, Mn, and Cu (also of LR) contents of FR; thus suggesting their role in improving the mineral nutrition of plants (Table 2). Moreover, the organic carbon contents of small macroaggregates positively correlated with the B, K, and S contents of LR, and the Mg, P, and K contents of FR in the soybean rhizosphere soil environment. The organic carbon contents of small macroaggregates negatively correlated with the Zn, Ca, and B contents, and the Ca, Mn, and S contents of FR and soybean shoots; which nevertheless suggest the immobilization role of organic carbon in nutrients [42] (Figure 4). The positive relationship of macroaggregate abundance and their organic carbon contents with belowground and aboveground plant traits could be due to improved microbial activities and other plant beneficial properties of the macroaggregates [43]. For instance, a recent study reported a positive correlation between the abundance of macroaggregates and length of the external mycorrhizal hyphae in the soil environment [44]. In another study, the abundance of macroaggregates positively correlated with the contents of phenolic compounds (phenols and phenolic acids), phospholipid fatty acids (PLFA), and soil organic matter [45]. Thus, our results and previous evidence suggest the strong role of soil macroaggregates and associated carbon in improving belowground and aboveground plant traits.

We also attempted to discern the relationship between mesoaggregates and belowground and aboveground plants, though these are less investigated with respect to plant growth than macroaggregates and microaggregates. The abundance of this aggregate-size class positively correlated with the P contents of the LR, though these demonstrated few correlations with other plant traits (Table 3). However, interestingly, the organic carbon contents of mesoaggregates demonstrated positive correlation with the S, B, and K contents of LR, with adaxial and abaxial trichome densities, with the P and K contents of LR, and with the B, S, and Mn contents of shoot tissues. Nevertheless, we also found a negative correlation between the organic carbon contents of mesoaggregates and the B, Zn, and Cu contents of LR (Figure 5). Recently, several studies have reported the significance of mesoaggregates in carbon sequestration, microbial activities, and microbial responses to soil and crop management practices, along with the impact of land use changes on mesoaggregates and their properties [46–48]. However, our study is the first to report that the relationship between important plant traits and mesoaggregates clearly predicts their significance in plant growth, nutrition, and fitness.

The evaluation of the relationship between soil microaggregates and plant traits showed that the abundance of soil microaggregates correlated positively with adaxial and abaxial trichome leaf density, the K and B contents of LR, and the Zn contents of LR. However, their organic carbon contents positively correlated with the S, B, and K contents of LR, and the P and K contents of LR. It is important to mention that abundance of microaggregates also positively correlated with major plant growth traits, such as root length and surface area (Figures 2 and 6), thus predicting the role of microaggregates and their properties in plant growth and nutrition [49]. However, their carbon contents showed a negative relationship with the B, Zn, and Cu contents of LR, and the B, S, and Mn contents of shoots (Figure 6). Though several studies have predicted the importance of microaggregates in biogeochemical processes related to soil fertility, health, and plant growth [45,50], little information is available about their role in plant growth, nutrition, and defense. For instance, some evidence suggests the proliferation and enlargement of hyphae between microaggregates, which could possibly improve plant root systems (Figure 2), though further research is needed to generalize the role of microaggregates in plant growth parameters and other fitness traits.

5. Conclusions and Limitations

In this study, we investigated the relationship between different soil aggregate-size classes (large macroaggregates, small macroaggregates, mesoaggregates, and microaggregates) and their organic carbon contents with several belowground and aboveground soybean plant traits. Our study revealed several novel and interesting relationships between soil structural properties and plant traits. Notably, the small macroaggregates represented a major portion of the rhizosphere soil system of the soybean plants, while organic carbon contents decreased with decreasing soil aggregate size. Only microaggregates showed a significant relationship with root architectural traits, such as length and surface area. The organic carbon contents of large macroaggregates showed correlation with several plant traits, such as trichome density and root and shoot mineral traits; whereas those of small macroaggregates and mesoaggregates showed correlation with fourteen plant traits related to leaf trichomes and root and shoot mineral contents. Interestingly, the organic carbon contents of microaggregates showed significant correlation with seventeen different aboveground and belowground plant traits. The abundance of small macroaggregates and organic carbon contents of microaggregates appeared to be key determinants of plant traits. Moreover, we observed that the organic carbon contents of different aggregate-size classes demonstrated positive correlations with leaf trichomes—an important plant defense trait, while microbial activities were often linked with soil carbon contents and the induction of leaf trichomes—though further research is needed to elucidate these interactions. Meanwhile, data showed that the improved mineral contents of different root fractions, such as fine and large roots, could be due to better aggregation of soil particles into different aggregate-size classes. Meanwhile, the widespread negative correlations between organic carbon contents of different soil aggregate-size classes and some mineral contents of root and shoots nevertheless suggest their role in nutrient sequestrations. Despite these interesting results, there are some limitations and precautions that need to be considered in interpreting our results and planning future research. First, though dry-sieving is widely used to characterize different soil aggregate-size classes and their properties, there might be some soil textural particles (sand, silt, clay) other than soil aggregates coming through the sieving process, so those need to be distinguished. Second, the rhizosphere soils of different agronomic crops might have different proportions or properties of soil aggregate-size classes and their relationships with the plant traits; thus, further research to needed to establish crop-specific relationships.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ecologies4010007/s1.

Author Contributions: Conceptualization, M.I.u.K., D.T. and M.S.; Methodology, D.T., A.A., J.B., A.N. and J.I.; Software, J.F.J., B.K.R. and M.S.; Validation, J.I. and M.S.; Formal analysis, M.I.u.K., J.F.J., A.A., J.B., A.N., J.I. and M.S.; Investigation, D.T., B.K.R. and M.S.; Resources, M.I.u.K., D.T., A.A. and M.S.; Data curation, M.I.u.K., A.A., J.B., A.N., J.I. and M.S.; Writing—Original draft, M.I.u.K. and M.S.; Writing—Review & editing, D.T., J.F.J., A.A., B.K.R., J.I. and M.S.; Visualization, J.F.J. and M.S.; Supervision, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We acknowledge the help of several undergraduate students and field workers during the preparation of this work. Moreover, we greatly appreciate the insightful comments, suggestions, and thoughts about interpretations provided by the referees, which certainly helped us improve the quality of this manuscript. Finally, we acknowledge funding from National Science Foundation (NSF-USA) under award 2100777.

Conflicts of Interest: The authors declare no competing interest.

References

1. Saleem, M.; Pervaiz, Z.H.; Contreras, J.; Lindenberger, J.H.; Hupp, B.M.; Chen, D.; Zhang, Q.; Wang, C.; Iqbal, J.; Twigg, P. Cover Crop Diversity Improves Multiple Soil Properties via Altering Root Architectural Traits. *Rhizosphere* **2020**, *16*, 100248. [CrossRef]
2. Topps, D.; Khabir, M.I.U.; Abdelmagid, H.; Jackson, T.; Iqbal, J.; Robertson, B.K.; Pervaiz, Z.H.; Saleem, M. Impact of Cover Crop Monocultures and Mixtures on Organic Carbon Contents of Soil Aggregates. *Soil Syst.* **2021**, *5*, 43. [CrossRef]
3. Fang, X.; Zhou, G.; Li, Y.; Liu, S.; Chu, G.; Xu, Z.; Liu, J. Warming Effects on Biomass and Composition of Microbial Communities and Enzyme Activities within Soil Aggregates in Subtropical Forest. *Biol. Fertil. Soils* **2016**, *52*, 353–365. [CrossRef]
4. Edwards, L.M. The Effect of Alternate Freezing and Thawing on Aggregate Stability and Aggregate Size Distribution of Some Prince Edward Island Soils. *J. Soil Sci.* **1991**, *42*, 193–204. [CrossRef]
5. Kou, T.J.; Zhu, P.; Huang, S.; Peng, X.X.; Song, Z.W.; Deng, A.X.; Gao, H.J.; Peng, C.; Zhang, W.J. Effects of Long-Term Cropping Regimes on Soil Carbon Sequestration and Aggregate Composition in Rainfed Farmland of Northeast China. *Soil Tillage Res.* **2012**, *118*, 132–138. [CrossRef]
6. Han, L.; Sun, K.; Jin, J.; Xing, B. Some Concepts of Soil Organic Carbon Characteristics and Mineral Interaction from a Review of Literature. *Soil Biol. Biochem.* **2016**, *94*, 107–121. [CrossRef]
7. da Silva, A.P.; Babujia, L.C.; Franchini, J.C.; Ralisch, R.; Hungria, M.; de Fátima Guimarães, M. Soil Structure and Its Influence on Microbial Biomass in Different Soil and Crop Management Systems. *Soil Tillage Res.* **2014**, *142*, 42–53. [CrossRef]
8. Wang, G.; Zhang, L.; Zhuang, Q.; Yu, D.; Shi, X.; Xing, S.; Xiong, D.; Liu, Y. Quantification of the Soil Organic Carbon Balance in the Tai-Lake Paddy Soils of China. *Soil Tillage Res.* **2016**, *155*, 95–106. [CrossRef]
9. Gregorich, E.G.; Liang, B.C.; Drury, C.F.; Ellert, B.H. Fertilization Effects on Physically Protected Light Fraction Organic Matter. *Soil Sci. Soc. Am. J.* **1997**, *61*, 482–484. [CrossRef]
10. Jiang, X.-J.; Xie, D.-T. Combining Ridge with No-Tillage in Lowland Rice-Based Cropping System: Long-Term Effect on Soil and Rice Yield. *Pedosphere* **2009**, *19*, 515–522. [CrossRef]
11. Zhang, S.; Li, Q.; Lü, Y.; Zhang, X.; Liang, W. Contributions of Soil Biota to C Sequestration Varied with Aggregate Fractions under Different Tillage Systems. *Soil Biol. Biochem.* **2013**, *62*, 147–156. [CrossRef]
12. Wang, X.; Qi, J.-Y.; Zhang, X.-Z.; Li, S.-S.; Latif Virk, A.; Zhao, X.; Xiao, X.-P.; Zhang, H.-L. Effects of Tillage and Residue Management on Soil Aggregates and Associated Carbon Storage in a Double Paddy Cropping System. *Soil Tillage Res.* **2019**, *194*, 104339. [CrossRef]
13. Rahman, M.T.; Guo, Z.C.; Zhang, Z.B.; Zhou, H.; Peng, X.H. Wetting and Drying Cycles Improving Aggregation and Associated C Stabilization Differently after Straw or Biochar Incorporated into a Vertisol. *Soil Tillage Res.* **2018**, *175*, 28–36. [CrossRef]
14. Pires, L.F.; Auler, A.C.; Roque, W.L.; Mooney, S.J. X-ray Microtomography Analysis of Soil Pore Structure Dynamics under Wetting and Drying Cycles. *Geoderma* **2020**, *362*, 114103. [CrossRef] [PubMed]
15. Diel, J.; Vogel, H.-J.; Schlüter, S. Impact of Wetting and Drying Cycles on Soil Structure Dynamics. *Geoderma* **2019**, *345*, 63–71. [CrossRef]
16. Bathke, G.R.; Cassel, D.K.; Hargrove, W.L.; Porter, P.M. Modification of Soil Physical Properties and Root Growth Response. *Soil Sci.* **1992**, *154*, 316–329. [CrossRef]
17. Letey, J. Relationship between Soil Physical Properties and Crop Production. In *Advances in Soil Science*; Stewart, B.A., Ed.; Advances in Soil Science; Springer: New York, NY, USA, 1985; pp. 277–294. ISBN 978-1-4612-5046-3.
18. Xiu, L.; Zhang, W.; Sun, Y.; Wu, D.; Meng, J.; Chen, W. Effects of Biochar and Straw Returning on the Key Cultivation Limitations of Albic Soil and Soybean Growth over 2 years. *Catena* **2019**, *173*, 481–493. [CrossRef]
19. Zhang, Q.; Li, S.; Saleem, M.; Ali, M.Y.; Xiang, J. Biochar and Earthworms Synergistically Improve Soil Structure, Microbial Abundance, Activities and Pyraclostrobin Degradation. *Appl. Soil Ecol.* **2021**, *168*, 104154. [CrossRef]
20. Kohler, J.; Caravaca, F.; Roldán, A. An AM Fungus and a PGPR Intensify the Adverse Effects of Salinity on the Stability of Rhizosphere Soil Aggregates of Lactuca Sativa. *Soil Biol. Biochem.* **2010**, *42*, 429–434. [CrossRef]
21. Reinhart, K.O.; Vermeire, L.T. Soil Aggregate Stability and Grassland Productivity Associations in a Northern Mixed-Grass Prairie. *PLoS ONE* **2016**, *11*, e0160262. [CrossRef]
22. Murungu, F.S.; Nyamugafata, P.; Chiduza, C.; Clark, L.J.; Whalley, W.R. Effects of Seed Priming, Aggregate Size and Soil Matric Potential on Emergence of Cotton (*Gossypium hirsutum* L.) and Maize (*Zea mays* L.). *Soil Tillage Res.* **2003**, *74*, 161–168. [CrossRef]
23. Nichols, K.A.; Toro, M. A Whole Soil Stability Index (WSSI) for Evaluating Soil Aggregation. *Soil Tillage Res.* **2011**, *111*, 99–104. [CrossRef]
24. Six, J.; Bossuyt, H.; Degryze, S.; Denef, K. A History of Research on the Link between (Micro)Aggregates, Soil Biota, and Soil Organic Matter Dynamics. *Soil Tillage Res.* **2004**, *79*, 7–31. [CrossRef]
25. Pervaiz, Z.H.; Iqbal, J.; Zhang, Q.; Chen, D.; Wei, H.; Saleem, M. Continuous Cropping Alters Multiple Biotic and Abiotic Indicators of Soil Health. *Soil Syst.* **2020**, *4*, 59. [CrossRef]
26. Adesemoye, A.; Pervaiz, Z.H.; Parikh, L.; Kodati, S.; Zhang, Q.; Stepanović, S.; Saleem, M. Rhizobacterial, Fusarium Complex, and Fungicide Seed Treatments Regulate Shoot and Root Traits of Soybean Plants. *J. Soil Sci. Plant Nutr.* **2021**, *21*, 3502–3513. [CrossRef]
27. Pervaiz, Z.H.; Contreras, J.; Hupp, B.M.; Lindenberger, J.H.; Chen, D.; Zhang, Q.; Wang, C.; Twigg, P.; Saleem, M. Root Microbiome Changes with Root Branching Order and Root Chemistry in Peach Rhizosphere Soil. *Rhizosphere* **2020**, *16*, 100249. [CrossRef]

28. Jiménez, J.J.; Lal, R.; Leblanc, H.A.; Russo, R.O.; Raut, Y. The Soil C Pool in Different Agroecosystems Derived from the Dry Tropical Forest of Guanacaste, Costa Rica. *Ecol. Eng.* **2008**, *34*, 289–299. [CrossRef]
29. Wang, B.; Brewer, P.E.; Shugart, H.H.; Lerdau, M.T.; Allison, S.D. Soil Aggregates as Biogeochemical Reactors and Implications for Soil–Atmosphere Exchange of Greenhouse Gases—A Concept. *Glob. Change Biol.* **2019**, *25*, 373–385. [CrossRef]
30. Sainju, U.M. Carbon and Nitrogen Pools in Soil Aggregates Separated by Dry and Wet Sieving Methods. *Soil Sci.* **2006**, *171*, 937–949. [CrossRef]
31. Kibblewhite, M.G.; Ritz, K.; Swift, M.J. Soil Health in Agricultural Systems. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 685–701. [CrossRef]
32. Menon, M.; Mawodza, T.; Rabbani, A.; Blaud, A.; Lair, G.J.; Babaei, M.; Kercheva, M.; Rousseva, S.; Banwart, S. Pore System Characteristics of Soil Aggregates and Their Relevance to Aggregate Stability. *Geoderma* **2020**, *366*, 114259. [CrossRef]
33. Al-Maliki, S.; Ebreesum, H. Changes in Soil Carbon Mineralization, Soil Microbes, Roots Density and Soil Structure Following the Application of the Arbuscular Mycorrhizal Fungi and Green Algae in the Arid Saline Soil. *Rhizosphere* **2020**, *14*, 100203. [CrossRef]
34. Jahangir, M.M.R.; Islam, S.; Nitu, T.T.; Uddin, S.; Kabir, A.K.M.A.; Meah, M.B.; Islam, R. Bio-Compost-Based Integrated Soil Fertility Management Improves Post-Harvest Soil Structural and Elemental Quality in a Two-Year Conservation Agriculture Practice. *Agronomy* **2021**, *11*, 2101. [CrossRef]
35. Qaswar, M.; Jing, H.; Ahmed, W.; Dongchu, L.; Shujun, L.; Lu, Z.; Cai, A.; Lisheng, L.; Yongmei, X.; Jusheng, G.; et al. Yield Sustainability, Soil Organic Carbon Sequestration and Nutrients Balance under Long-Term Combined Application of Manure and Inorganic Fertilizers in Acidic Paddy Soil. *Soil Tillage Res.* **2020**, *198*, 104569. [CrossRef]
36. Rillig, M.C.; Muller, L.A.; Lehmann, A. Soil Aggregates as Massively Concurrent Evolutionary Incubators. *ISME J.* **2017**, *11*, 1943–1948. [CrossRef]
37. Rillig, M.C.; Aguilar-Trigueros, C.A.; Bergmann, J.; Verbruggen, E.; Veresoglou, S.D.; Lehmann, A. Plant Root and Mycorrhizal Fungal Traits for Understanding Soil Aggregation. *New Phytol.* **2015**, *205*, 1385–1388. [CrossRef] [PubMed]
38. Chen, Y.; Ren, K.; Su, J.; He, X.; Zhao, G.; Hu, B.; Chen, Y.; Xu, Z.; Jin, Y.; Zou, C. Rotation and Organic Fertilizers Stabilize Soil Water-Stable Aggregates and Their Associated Carbon and Nitrogen in Flue-Cured Tobacco Production. *J. Soil Sci. Plant Nutr.* **2020**, *20*, 192–205. [CrossRef]
39. Wright, A.L.; Hons, F.M. Soil Carbon and Nitrogen Storage in Aggregates from Different Tillage and Crop Regimes. *Soil Sci. Soc. Am. J.* **2005**, *69*, 141–147. [CrossRef]
40. Li, Y.; Xie, Z.; Zhou, M.; Liu, C.; Yu, Z.; Wu, J.; Jin, J.; Chen, Y.; Zhang, X.; Liu, X. Soybean Yield and Quality Relative to Mollisols Fertility with 7-Year Consecutive Cattle Manure Application under Maize-Soybean Rotation. *Land Degrad. Dev.* **2021**, *32*, 4740–4754. [CrossRef]
41. Anothai, J.; Chairin, T. Soil Physicochemical Properties Closely Associated with Fungal Enzymes and Plant Defense Enzymes in Ganoderma-Infected Oil Palm Orchards. *Plant Soil* **2020**, *456*, 99–112. [CrossRef]
42. El-Naggar, A.; Lee, M.-H.; Hur, J.; Lee, Y.H.; Igalavithana, A.D.; Shaheen, S.M.; Ryu, C.; Rinklebe, J.; Tsang, D.C.W.; Ok, Y.S. Biochar-Induced Metal Immobilization and Soil Biogeochemical Process: An Integrated Mechanistic Approach. *Sci. Total Environ.* **2020**, *698*, 134112. [CrossRef] [PubMed]
43. Jiang, Y.; Zhou, H.; Chen, L.; Yuan, Y.; Fang, H.; Luan, L.; Chen, Y.; Wang, X.; Liu, M.; Li, H.; et al. Nematodes and Microorganisms Interactively Stimulate Soil Organic Carbon Turnover in the Macroaggregates. *Front. Microbiol.* **2018**, *9*, 2803. [CrossRef] [PubMed]
44. Miller, R.M.; Jastrow, J.D. The Role of Mycorrhizal Fungi in Soil Conservation. In *Mycorrhizae in Sustainable Agriculture*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 1992; pp. 29–44. ISBN 978-0-89118-320-4.
45. Xiong, L.; Liu, X.; Vinci, G.; Sun, B.; Drosos, M.; Li, L.; Piccolo, A.; Pan, G. Aggregate Fractions Shaped Molecular Composition Change of Soil Organic Matter in a Rice Paddy under Elevated CO2 and Air Warming. *Soil Biol. Biochem.* **2021**, *159*, 108289. [CrossRef]
46. Singh, M.K.; Singh, S.; Ghoshal, N. Impact of Land Use Change on Soil Aggregate Dynamics in the Dry Tropics. *Restor. Ecol.* **2017**, *25*, 962–971. [CrossRef]
47. Cavalcante, D.M.; de Castro, M.F.; Chaves, M.T.L.; da Silva, I.R.; de Oliveira, T.S. Effects of Rehabilitation Strategies on Soil Aggregation, C and N Distribution and Carbon Management Index in Coffee Cultivation in Mined Soil. *Ecol. Indic.* **2019**, *107*, 105668. [CrossRef]
48. Zhong, Z.; Wu, S.; Lu, X.; Ren, Z.; Wu, Q.; Xu, M.; Ren, C.; Yang, G.; Han, X. Organic Carbon, Nitrogen Accumulation, and Soil Aggregate Dynamics as Affected by Vegetation Restoration Patterns in the Loess Plateau of China. *Catena* **2021**, *196*, 104867. [CrossRef]
49. Totsche, K.U.; Amelung, W.; Gerzabek, M.H.; Guggenberger, G.; Klumpp, E.; Knief, C.; Lehndorff, E.; Mikutta, R.; Peth, S.; Prechtel, A.; et al. Microaggregates in Soils. *J. Plant Nutr. Soil Sci.* **2018**, *181*, 104–136. [CrossRef]
50. Edwards, A.P.; Bremner, J.M. Microaggregates in Soils1. *J. Soil Sci.* **1967**, *18*, 64–73. [CrossRef]