Short-term effects of biological and physical forces on aggregate formation in soils with different clay mineralogy

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Abstract

The mechanisms resulting in the binding of primary soil particles into stable aggregates vary with soil parent material, climate, vegetation, and management practices. In this study, we investigated short-term effects of: (i) nutrient addition (Hoagland's solution), (ii) organic carbon (OC) input (wheat residue), (iii) drying and wetting action, and (iv) root growth, with or without dry–wet cycles, on aggregate formation and stabilization in three soils differing in weathering status and clay mineralogy. These soils included a young, slightly weathered temperate soil dominated by 2:1 (illite and chlorite) clay minerals; a moderately weathered soil with mixed [2:1 (vermiculite) and 1:1 (kaolinite)] clay mineralogy and oxides; and a highly weathered tropical soil dominated by 1:1 (kaolinite) clay minerals and oxides. Air-dried soil was dry sieved through a 250 μm sieve to break up all macroaggregates and 100 g-subsamples were brought to field capacity and incubated for 42 days. After 14 and 42 days, aggregate stability was measured on field moist and air-dried soil, to determine unstable and stable aggregation respectively. In control treatments (i.e., without nutrient or organic matter addition, without roots and at constant moisture), the formation of unstable and stable macroaggregates (> 250 μm) increased in the order: 2:1 clay soil < mixed clay soil < 1:1 clay soil. After 42 days of incubation, nutrient addition significantly increased both unstable and stable macroaggregates in the 2:1 and 1:1 clay soils. In all soils, additional OC input increased both unstable and stable macroaggregate formation. The increase in macroaggregation with OC input was highest for the mixed clay soil and lowest for the 1:1 clay soil. In general, drying and wetting cycles had a positive effect on the formation of macroaggregates. Root growth caused a decrease in unstable macroaggregates in all soils. Larger amounts of macroaggregates were found in the mixed clay and oxides soil when plants were grown under 50% compared to 100% field capacity conditions. We concluded that soils dominated by variable charge clay minerals (1:1 clays and oxides) have higher potential to form stable aggregates when OC concentrations are low. With additional OC inputs, the greatest response in stable macroaggregate formation occurred in soils with mixed mineralogy, which is probably a result of different binding mechanisms occurring: i.e., electrostatic bindings between 2:1 clays, 1:1 clays and oxides (i.e. mineral-mineral bindings), in addition to OM functioning as a binding agent between 2:1 and 1:1 clays.

Abbreviations: FDW – fast drying-slow rewetting; SDW – slow drying-slow rewetting; FC – field capacity; OC – organic carbon; SOM – soil organic matter; ZPC – zero point of charge

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Introduction

Soil structure (e.g., aggregation) controls a range of soil properties such as aeration, water infiltration and drainage, which influence crop establishment and growth while also providing habitat for soil biota. Maintenance of aggregate stability is important to prevent structural losses when soil is subjected to mechanical stresses (e.g. tillage) or climatic influences (e.g. rainfall, freeze–thaw cycles). The rate at which aggregates can be reformed and restabilized through natural processes, amendments, and cultivation is largely influenced by soil properties (e.g. organic matter content, clay mineralogy, soil texture). Several authors have suggested that fresh residue forms the nucleation center for aggregate formation (Jastrow, 1996; Puget et al., 1995; Six et al., 1998) through the production of microbial-derived substances that bind residue and soil particles into aggregates. Consequently, enhanced microbial activity (e.g. through organic matter input or nutrient addition) should stimulate aggregate formation. The importance of organic matter as the primary binding agent for soil aggregates holds true for moderately weathered soils dominated by 2:1 clay minerals, where the negative surface charges of soil organic matter (SOM) and clay minerals are mutually bound to positively charged polyvalent metal cations (Edwards and Bremner, 1967). However, in soils exposed to more intense weathering regimes, 1:1 clay minerals and oxides dominate, which have both positive and negative surface charges at field pH, and have a greater capacity for mineral–mineral binding (Oades and Waters, 1991; Six et al., in press). Therefore, researchers have suggested that aggregation is less related to SOM in highly weathered soils (Six et al., 2000b; Tisdall and Oades, 1982).

The effects of drying and wetting on soil structure are still unclear, because both increases and decreases in water-stable aggregation have been observed following drying and subsequent rewetting. Drying can enhance the formation of intermolecular associations between organic molecules and mineral surfaces (Kemper and Rosenau, 1984) and the sorption of oxides on clay domain surfaces (Schahabi and Schwertmann, 1970). On the other hand, upon rewetting, slaking (i.e. aggregate disruption under the action of air and water) can offset the stabilizing effect of drying. The balance between these counteracting processes is considerably influenced by soil properties, such as clay mineralogy, oxide content, texture, OM content (Caron et al., 1992; Haynes and Swift, 1990).

The contribution of roots to aggregate dynamics is variable and not fully elucidated. Roots can enhance aggregate formation and stabilization directly by physically enmeshing soil particles and producing organic cementing agents (root exudates). Indirectly, roots can stimulate microbial biomass, which synthesizes polymers that act as binding agents (Jastrow et al., 1998; Tisdall and Oades, 1979). Different effects of root growth on aggregation have been attributed to: (1) variation in labile organic C inputs (Reid and Goss, 1981), (2) chemical disruption of aggregates by destroying SOM-cation-clay linkages due to chelating agents released into the rhizosphere (Reid et al., 1982), (3) aggregate penetration and mechanical disruption (Materechera et al., 1994), (4) cohesion by soil drying (Caron et al., 1992; Tisdall and Oades, 1979), (5) mycorrhizal symbiosis (Miller and Jastrow, 1990; Tisdall and Oades, 1979), and (6) differences in root architecture and morphology (Miller and Jastrow, 1990).

The objective of this study was to evaluate short-term effects of nutrient addition, OC inputs, active root growth, and drying and wetting cycles on macroaggregate formation in soils differing in weathering status (i.e. clay mineralogy and oxide content). We hypothesized that: (1) if nutrients are limiting for microbial activity in the soils, nutrient addition would enhance macroaggregate formation; (2) larger OC inputs would stimulate microbial activity and subsequently increase macroaggregate formation in all types of soil, but to a greater extent in soils with 2:1 clay minerals; and (3) active root growth would enhance macroaggregate formation and stabilization.

Materials and methods

Soils and sampling

Surface soil (approximately 0–10 cm) was collected from three long-term agricultural field experiments in March 2000. The three sites are located near Sidney, NE (41° 14′ N, 103° 00′ W), Lexington, KY (38° 07′ N, 84° 29′ W), and Passo Fundo, Brazil (28° 15′ S, 52° 24″ W). All three sites include annual crops under conventional tillage since 1970 (Sidney and Lexington) and 1985 (Passo Fundo). Detailed descriptions of the sites and experiments are reported for Sidney (Lyon et al., 1997), Lexington (Frye and Blevins, 1997) and Passo Fundo (Santos et al., 1995). General soil characteristics are given in Table 1. After collection, the

Table 1. General soil characteristics

Site	Sidney, NE 2:1	Lexington, KE mixed	Passo Fundo, Brazil 1:1
Soil classification (USDA)	Pachic Haplustoll	Typic Paleudalf	Latosol-Oxisol
Texture (g 100 g^{-1} soil)	Loam	Silty clay loam	Clay
Clay	27	12	59
Silt	33	82	15
Sand	40	6	26
CEC [a] (cmol$_c$ kg^{-1} clay)	53.9	82.9	16.2
MAT [b] (°C)	8.5	13.1	17.5
MAP [c] (mm)	440	1127	1788
PH (H$_2$O)	6.1	6.7	5.3
Organic carbon (g 100 g^{-1} soil)	1.35	1.26	1.64
Mineralogy	[d] Illite, (hepta)-chlorite (cronstedtite), feldspar, quartz	[d] Kaolinite, vermiculite, illite, feldspar, quartz	Kaolinite, quartz

[a] CEC, cation exchange capacity (silverthioureum (AgTU) method); [b] MAT, mean annual air temperature; [c] MAP, mean annual precipitation; [d] adopted from Six et al. (2000b).

soil was air-dried for 2 days (moisture content after air–drying was 1–2%) and pushed through a 250 μm sieve to break up all macroaggregates ($> 250 \mu$m) into $< 250 \mu$m sized microaggregates and silt plus clay particles. The 250–2000 μm sized sand and particulate organic matter (POM) fractions were kept and remixed with the soil after sieving. The air-dried 250 μm sieved soil was thoroughly mixed; soil samples of approximately 100 g were taken and mixed with 500–1000 μm sized wheat residue (stems and leaves; C/N = 96) in amounts according to the treatments described below. Samples were then packed into 5 cm diameter aluminum cylinders, resulting in a soil bulk density of approximately 1.2 g cm^{-3}. Field capacity was measured on three replicates of 50 g air-dried soil, which averaged $26.4 \pm 1.5\%$; $21.1 \pm 0.3\%$; and $27.2 \pm 0.4\%$ (g H$_2$O 100 g^{-1} air-dried soil) for Sidney, Lexington and Passo Fundo, respectively.

General soil characteristics analyses

Soil texture was determined by shaking 30-g soil samples in 5% sodium hexametaphosphate solution for 18 h. Sand content was measured by sieving (>53 μm). Clay content was determined by density with a hydrometer. Silt was calculated by difference. Cation exchange capacity (CEC) was determined using the silverthioureum (AgTu) method. This method relies on a very high selectivity of the exchange surface for the silverthioureum-complex and allows CEC measurements at low ionic strength (0.01 m) and at prevailing soil pH value (Pleysier and Juo, 1980). Tropical soils have mineral particles with variable (pH dependent) charges and thus CEC should be measured at field pH. Total soil organic C and N concentrations were measured on ground soil subsamples with a LECO-CHN-1000 analyzer (Leco Corp., St. Joseph, MI). Soil pH was measured in a 2:1 water: soil solution with a digital pH meter.

Clay mineralogy was previously analyzed by Six et al. (2000b) for the Sidney and Lexington soil. The mineralogy for the Passo Fundo soil was similarly determined through X-ray diffraction analysis. Three replicates of 50 g were taken from the 250 μm sieved soil, mixed with water to give a 1:1 soil-to-water ratio. The soil–water suspension was treated with 30% H$_2$O$_2$ at 60–70 °C. H$_2$O$_2$ was added in small amounts until the sample ceased to froth. Then, the samples were heated for several hours to evaporate excess H$_2$O$_2$. The samples were rinsed with deionized water and shaken for 18 h to disperse the soil completely. The <20 μm fraction was isolated by sieving and sus-

Table 2. Amounts of Fe, Al, and Si sequentially extracted by citrate-ascorbic acid (Fe$_{Ca}$, Al$_{Ca}$, Si$_{Ca}$) and citrate-dithionite (Fe$_d$, Al$_d$, Si$_d$)

Site	Fe$_{Ca}$	Al$_{Ca}$	Si$_{Ca}$ (g kg^{-1})	Fe$_d$	Al$_d$	Si$_d$
Sidney, NE	0.46 ± 0.05	0.70 ± 0.14	0.82 ± 0.06	2.05 ± 0.07	0.36 ± 0.00	1.14 ± 0.06
Lexington, KY	7.25 ± 1.63	2.01 ± 0.21	0.28 ± 0.06	6.68 ± 0.26	0.69 ± 0.10	0.24 ± 0.03
Passo Fundo, Brazil	4.34 ± 0.28	1.34 ± 0.06	0.28 ± 0.09	21.86 ± 0.93	2.17 ± 0.16	0.35 ± 0.04

pended in 250 mL deionized water. Oriented samples for X-ray diffraction were made by the Millipore filter transfer method (Moore and Reynolds, 1997). Non-crystalline components and 'free' sesquioxides were determined on all three soils. Noncrystalline components were determined with the citrate-ascorbic acid (CA) method described by Reyes and Torrent (1998). Briefly, 250 mg H$_2$O$_2$ treated whole soil was weighed out in centrifuge tubes and 50 ml 0.2 M sodium citrate – 0.05 M sodium ascorbic acid solution (pH=6) was added. Samples were shaken for 16 h and centrifuged. The supernatant was analyzed for Fe, Al, and Si by atomic absorption spectrophotometry. The residue of the CA extraction was used for the determination of 'free' sesquioxides with dithionite (Blakemore et al., 1987). One g sodium dithionite and 50 ml of 0.75 M sodium citrate were added to the residue and shaken for 16 h. The suspension was centrifuged and Fe, Al, and Si concentrations in the supernatant were determined by atomic absorption spectrophotometry. Data on these soil characteristics can be found in Table 2.

Experimental design

Soil samples were incubated for 42 days with the following treatments: (i) with or without extra nutrients; (ii) low or high input of organic carbon (OC); (iii) fast or slow drying–slow rewetting; and (iv) with or without plant and root growth. In treatments with plant growth, samples were incubated (v) with or without exposure to slow drying–slow rewetting (Table 3). In treatment (i), soil samples were mixed with 0.3 g wheat residue per 100 g soil and brought to field capacity (FC) with deionized water or nutrient solution (Hoagland's solution) containing Ca, N, K, Mg, P, Na and micronutrients (B, Mn, Zn, Cu, and Mo). Because the three soils have different moisture contents at FC, we added a same initial amount of nutrient solution to all soils (21 ml 100 g^{-1} soil). Deionized water was added for all additional moisture necessary

to reach FC. Samples were kept at FC during the entire experiment by incubating the cylinders with the soil samples in sealed glass jars with water-filled scintillation vials. The sealed glass jars had aluminum lids with luer lock fittings to allow collection of headspace gas samples for respiration measurements. Every 2 days, soil weight was measured and water was added when soil moisture losses were greater than 0.5 ml 100 g^{-1} soil. In treatment (ii), the low OC input level (i.e. 0.3 g wheat residue 100 g^{-1} soil) was based on the amount that would be incorporated under field conditions in Sidney, NE (Lyon et al., 1997). For the high OC input level, soil samples were mixed with 2 g wheat residue per 100 g soil. Soil samples of both low and high OC input treatments were brought to FC with nutrient solution, due to concern of nutrient limitation with high OC input. Soil samples in both the fast and slow drying–slow rewetting treatment (iii) were incubated at low OC and low nutrient input levels. For the fast drying–slow rewetting treatment (FDW), soil samples were incubated at FC in glass jars. Every two weeks, samples were taken out of the jars and treated as follows: soil was kept in the core, air-dried at 25 °C with a fan for 2 days (moisture content after air–drying was 1–2%), and slowly rewetted by slowly adding the amount of water to the soil required to attain FC. For the slow drying–slow rewetting treatment (SDW), soil samples were incubated in open air (i.e. outside jars) and therefore continuously exposed to natural air-drying. Every 2 days, moisture loss was measured by weight. When 50% of the moisture content at FC was reached, water was slowly added to bring the soil samples back to FC. These two dry–wet treatments were compared with the low nutrient, FC treatment to evaluate the effect of different drying rates on aggregation.

In treatment (iv), spring wheat seeds (1 seed per core) were put in the top layer of the soil samples mixed with wheat residue at low OC input level, and brought to FC with nutrient solution (Hoagland's solu-

Table 3. Summary of treatments in experimental design

Treatment code	Nutrients	OC input	Moisture	Root growth
− N (control)	None	0.3 g	constant FC	none
+ N	+ Hoagland's solution	0.3 g	constant FC	none
high OC	+ Hoagland's solution	2 g	constant FC	none
FDW	None	0.3 g	constant FC; every 14 days: fast drying–slow rewetting	none
SDW	None	0.3 g	open air; slow drying–slow rewetting at 50% FC	none
P-FC	+ Hoagland's solution	0.3 g	constant FC	yes
P-SDW	+ Hoagland's solution	0.3 g	open air; slow drying–slow rewetting at 50% FC	yes

tion). The samples were all incubated in open air in a greenhouse to have enough light for sufficient plant growth. In one set of soil samples (P-FC), FC was maintained by covering the soil with parafilm, minimizing air-drying and soil evaporation. When plants germinated, a hole was made through which the plant could grow. The other set (P-SDW) was continuously exposed to natural air-drying as for the SDW treatment. By comparing P-FC and P-SDW treatments versus FC and SDW treatments without plant growth, we could investigate both mechanical and drying effects of roots on aggregation. We hypothesized that dry–wet cycles would occur more frequently in the P-SDW compared to the SDW (−plants) treatment, due to plant transpiration.

Aggregate size distribution analyses

At days 14 and 42, three replicates of treatments (i), (ii) and (iii) were sampled and analyzed for aggregate size distribution. Aggregate size distribution was only measured at day 42 for treatment (iv), because of insufficient plant growth after 14 days. Field moist soil samples were taken from the cylinders and gently broken to pass an 8-mm sieve. Aggregate size separations and soil stability assessments were done by a wet-sieving method adapted from Elliott (1986). Half of the 8 mm sieved soil was wet-sieved at field capacity to measure 'unstable' aggregation, the other half was air-dried for 2 days and wet-sieved to measure 'stable' (i.e., slaking resistant) aggregation. Upon slaking, a higher amount of disruptive energy occurs because rapid wetting of dry soil leads to an entrapment of air and a build up of air pressure within the aggregates (Kemper and Rosenau, 1984). Wet-sieving of field moist soil is less disruptive and more relatively

'unstable' aggregates survive this treatment compared to slaking. A series of three sieves was used to obtain four aggregate size fractions: (1) >2000 μm (large macroaggregates); (2) 250–2000 μm (small macroaggregates); (3) 53–250 μm (microaggregates); and (4) <53 μm (silt and clay fraction). The soil samples (field moist or air-dry) were submerged in room temperature water on top of the 2000 μm sieve for 5 min prior to sieving. During sieving, aggregates were separated by manually moving the sieve up and down 3 cm with 50 repetitions during a period of 2 min. Water plus soil that went through the sieve was poured onto the next smaller sized sieve and the same sieving procedure was repeated. The aggregate fractions retained on each sieve were oven dried (50 °C) for 24 h, weighed and stored in glass jars at room temperature (21 °C).

Because sand of the same size as the aggregate size class (=aggregate-sized sand) is unlikely to be a part of an aggregate, it is necessary to correct for the aggregate-sized sand content (Elliott et al., 1991). This is important in our experiment, where we compare aggregation across soils with different textures. Therefore, a 5 g subsample was taken from the large macroaggregate, small macroaggregate, and microaggregate fractions obtained after wet–sieving, and dispersed in 0.5% sodium hexametaphosphate by shaking for 18 h on a reciprocal shaker. Sand-corrected aggregation was determined as follows:

$$\% \text{ sand corrected aggregates} = \frac{(\text{weight}_{\text{aggregate size class}} - \text{weight}_{\text{sand of the same size as aggregate size class}}) * 100}{\sum(\text{all fractions})_{\text{sand corrected weights}}}$$

After sand correction of all aggregate fractions, the sum of large and small macroaggregates was taken and compared across treatments and soils. This was done because no stable large macroaggregates were formed after 6 weeks of incubation and no significant differences in aggregate dynamics were noticed between large and small unstable macroaggregates.

Statistical analyses

When investigating the effect of OC input and nutrient addition on aggregate formation, two levels of OC and nutrients, three soils and two sampling days were used. For the investigation of the effect of dry–wet cycles and active root growth, five treatments and three soils were used. The measurements were subjected to ANOVA with the MIXED procedure of the SAS system (SAS/STAT, SAS Institute 1990). Treatment, soil and time were considered fixed effects while replicate was considered as a random effect ($n = 3$). Differences ($\alpha = 0.05$) between means were further tested with the DIFF option of the LSMEANS statement. In the experiments where the differences in aggregation due to OC input or nutrient addition were compared among soils, differences in means were tested between each combination of two soils by using the LSMEANS and ESTIMATE statements and Bonferroni's significant difference at a level of $P < 0.05/3$ (i.e., α/g with g the number of linear combinations).

Results

Soil characteristics

The three soils (Sidney, NE; Lexington, KY; and Passo Fundo, Brazil) differ in weathering degree and clay mineralogy. The Sidney soil is a lightly weathered temperate soil, dominated by 2:1 clay minerals (illite and chlorite) with a CEC of around 53.9 $cmol_c$ kg^{-1}clay (Table 1). The moderately weathered soil from Lexington has a mixed [2:1 and 1:1] clay mineralogy (vermiculites and kaolinites). In addition, this soil contains large amounts of Fe and Al extracted by citrate-ascorbic acid (Fe_{Ca}, Al_{Ca}) and dithionite (Fe_d, Al_d) (Table 2), indicating a substantial amount of amorphous and poorly crystalline oxides, which may play an important role in aggregate stability. Lexington soil was also characterized by a CEC of around 82.9 $cmol_c$ kg^{-1}clay, which is rather high for the amount of kaolinites found in this soil. This high CEC is attributed to the presence of vermiculites (Table 1),

which have some of the largest CEC of all minerals (Alexiades and Jackson, 1965). The soil from Passo Fundo is a highly weathered soil with a CEC of approximately 16.2 $cmol_c$ kg^{-1}clay. This weathered soil is dominated by 1:1 clay minerals (kaolinites) and has the highest amount of Fe- and Al-sesquioxides (Fe_d, Al_d) (Table 2). Throughout the text, the temperate soil from Sidney, the moderately weathered soil from Lexington, and the highly weathered soil from Passo Fundo will be referred to as the 2:1, mixed and 1:1 soil respectively, based on the predominant clay mineralogy.

Effect of nutrient addition

Without addition of nutrients, a general increase in unstable and stable macroaggregation was observed with increasing weathering degree, from 2:1 soil < mixed soil < 1:1 soil (Figure 1a). From days 14 to 42, unstable and stable macroaggregation did not change in the 1:1 soil. Unstable and stable macroaggregation in the mixed soil, and stable macroaggregation in the 2:1 soil significantly decreased after day 14. In the treatment with nutrients added, unstable macroaggregate formation increased over time (Figure 1b). During the first 14 days of incubation, nutrient addition caused a significant decrease in unstable macroaggregation in the mixed soil (Figure 1c). After 42 days of incubation, we observed a significant increase in unstable macroaggregates due to nutrient addition for both 2:1 and 1:1 soils. Nutrient addition positively affected stable macroaggregate formation in the 2:1 and 1:1 soils (Figure 1c).

Effect of organic carbon (OC) input

A relative increase in microbial activity (measured as respiration) was noticed due to OC input (data not shown). This increase was similar for all three soils and was the highest during the first 4 days of incubation.

Larger OC inputs significantly increased unstable and stable macroaggregate formation in all three soils (Figure 2b,c). Similar to the treatment with low OC input, highest amounts of unstable macroaggregates in the high OC treatment were found in the 1:1 soil (Figure 2a,b). The largest amounts of stable macroaggregates in the low OC treatment were observed in the 1:1 soil. With high OC input, the highest amounts of stable macroaggregates were formed in the mixed soil, both at days 14 and 42 (Figure 2b). Relative increases in unstable and stable macroaggregation due to OC
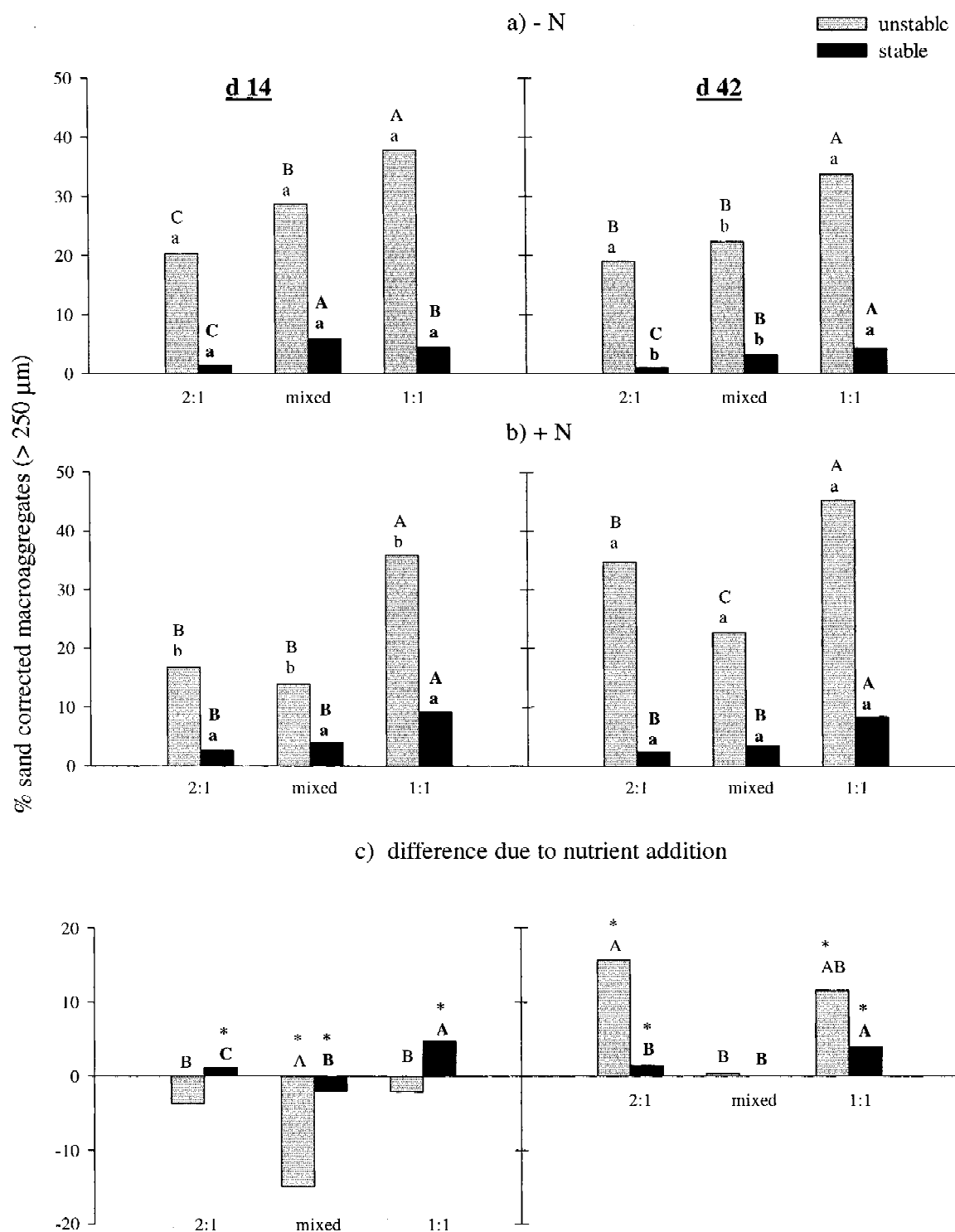
Figure 1. Effect of nutrient addition on unstable and stable macroaggregation (% sand corrected macroaggregates > 250 μm) after 14 and 42 days of field moist incubation with low input of OC (0.3 g wheat straw 100 g^{-1} soil). 2:1 = temperate soil (Sidney) dominated by 2:1 clay minerals; mixed = moderately weathered soil (Lexington) with mixed [2:1 and 1:1] clay minerals and oxides; 1:1 = highly weathered tropical soil (Passo Fundo) dominated by 1:1 clay minerals and oxides; −/+ N = without or with addition of Hoagland's solution prior to incubation. Values followed by a different uppercase letter within the same treatment/day/stability and among soils are significantly different (*P* < 0.05). Values followed by a different lowercase letter within the same treatment/soil/stability and between sampling days are significantly different (*P* < 0.05). In (c), * indicates that the value is significantly different from 0 (*P* < 0.05); values followed by a different uppercase letter within the same day/stability and among soils are significantly different (*P* < 0.05/3).

input varied in the three soils as follows: 1:1 soil $\leq$ 2:1 soil < mixed soil (Figure 2c).

Effect of drying and wetting forces

Examining unstable and stable macroaggregation in control soil samples, a similar pattern to Figure 1 was noticed: macroaggregation increased with increasing weathering degree: 2:1 soil < mixed soil <1:1 soil, at both day 14 (Figure 3) and day 42 (Figure 4). At day 14, the fast drying–slow rewetting (FDW) treatment caused a significant increase in unstable macroaggregation in all three soils (Figure 3a). Similar increases in unstable macroaggregation were noticed in the slow drying–slow rewetting (SDW) treatment at day 14. After 42 days, unstable macroaggregation was higher with both FDW and SDW compared to the control in the mixed soil (Figure 4a). In the 2:1 soil, SDW increased unstable macroaggregation, but FDW had no effect. Both FDW and SDW had no effect on unstable macroaggregation in the 1:1 soil. Stable macroaggregation was not affected by FDW or SDW at day 14 (Figure 3b). However, after 42 days, FDW and SDW had a positive effect on stable macroaggregation, but only in the mixed soil (Figure 4b).

Effect of active root growth

Unstable macroaggregation was significantly reduced in the presence of growing wheat plants in all three soils (Figure 4a). Air-drying in addition to plant growth seemed to have a counteracting effect on aggregation in the mixed clay and oxides soil, because larger amounts of macroaggregates were found in this soil when plants were grown in open air (SDW) compared to when plants were grown under FC conditions (Figure 4a). No significant changes with plant growth were observed in the mixed and 1:1 clay soils for stable macroaggregation compared to control soil after 6 weeks of incubation. However, active root growth caused a significant increase in stable macroaggregation in the 2:1 clay soil (Figure 4b).

Discussion

Effect of nutrients

In all three soils, nutrients had a positive effect on stimulating unstable macroaggregate formation over a longer time: where nutrients were added, macroaggregation increased over time, whereas in the

treatment without nutrients, maximum in macroaggregation was reached after 14 days (Figure 1). At day 14, stable macroaggregation was significantly higher in the treatment with nutrients compared to the treatment without nutrients in the 2:1 and 1:1 soils. In the mixed soil however, adding nutrients decreased macroaggregation at day 14 compared with the no nutrient treatments (Figure 1c). Interestingly, soil respiration was not affected by nutrient treatments in all soils during the entire incubation period (data not shown), suggesting that overall microbial activity in these soils was not limited by nutrients but also not stimulated by the addition of extra nutrients. It remains unclear why: (i) nutrient addition stimulated aggregate formation over time, (ii) a significant decrease in macroaggregation was only noticed in the mixed soil, whereas the 2:1 and 1:1 soils showed an increase in macroaggregation upon nutrient addition, and (iii) no significant changes in total microbial activity were noticed upon nutrient addition in any of these soils. We hypothesize that addition of nutrients can enhance ionic strength of the soil solution and decrease soil pH. This can alter electrostatic interactions between clay minerals, oxides and organic matter compounds, which might result in different changes in macroaggregation in soils with different clay mineralogy. However, not enough data is provided by this study to explain the observed differences in more detail.

Effect of OC input

Addition of extra OC significantly enhanced macroaggregate formation and stabilization in all three soils (Figure 2). This could be attributed to a larger production of microbial binding agents because microbial activity was significantly enhanced by addition of extra OC during the entire incubation period (data not shown). Aggregate formation can be of mechanical, electrostatic or biological nature. The majority of biologically formed bonds are caused by microorganisms through the production of polysaccharide 'gums'. Several researchers have reported that addition of readily available substrate causes a rapid stimulation of the soil microflora and this is accompanied by an increase in aggregate stability (Lynch and Bragg, 1985).

With high OC input, stable macroaggregation was still increasing over time between days 14 and 42 for the 2:1 and the mixed soils, whereas in the low OC treatment, maximum aggregation was already reached after 14 days. This suggests that the maximum capacity of a soil to form stable macroaggregates can
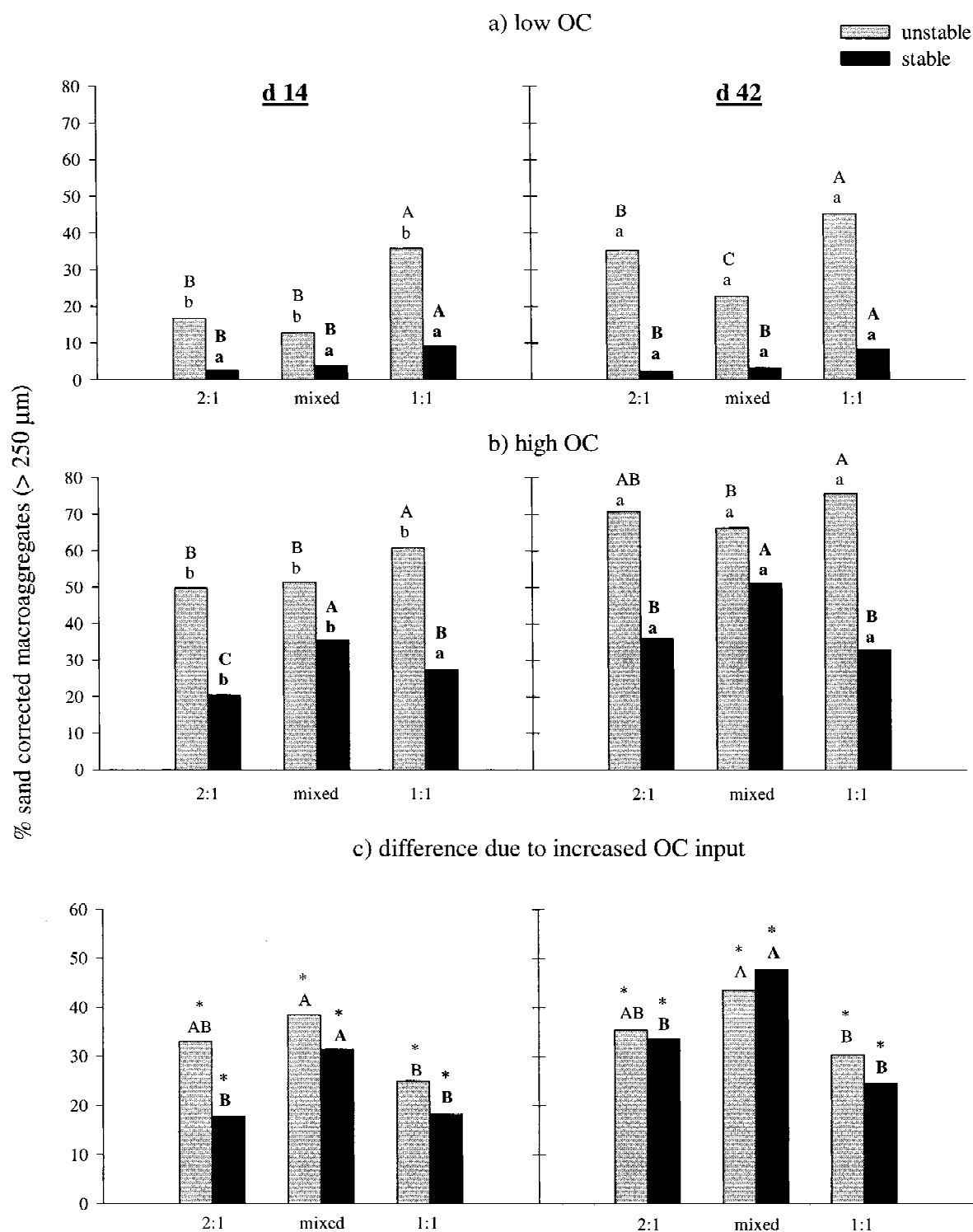
a) low OC

b) high OC

c) difference due to increased OC input

Figure 2. Effect of organic carbon input on unstable and stable macroaggregation (% sand corrected macroaggregates > 250 μm) after 14 and 42 days of field moist incubation (with addition of nutrients prior to incubation). Low OC = addition of 0.3 g wheat straw 100 g^{-1} soil; high OC = addition of 2 g wheat straw 100 g^{-1} soil. Axis labels as in Figure 1. Values followed by a different uppercase letter within the same treatment/day/stability and among soils are significantly different (P < 0.05). Values followed by a different lowercase letter within the same treatment/soil/stability and between sampling days are significantly different (P < 0.05). In (c), * indicates that the value is significantly different from 0 (P < 0.05); values followed by a different uppercase letter within the same day/stability and among soils are significantly different (P < 0.05/3).

be controlled by OC inputs in these soils. The 1:1 soil seemed to reach this level of maximum stable aggregation after 14 days in both low and high OC treatments. This suggests that the 1:1 soil is characterized by a faster aggregate stabilization rate compared to the other two soils and that this rate of aggregate stabilization is not affected by OC input.

Effect of drying and wetting forces

Both drying and wetting treatments (FDW and SDW) had a positive effect on the formation of unstable macroaggregates in all three soils after 14 days of incubation. Repeated dry–wet cycles have been reported to increase the stabilization of aggregates (Denef et al., 2001; Utomo and Dexter, 1982). Other studies have noted that soils become completely structure-less after repeated dry–wet cycles (Jager and Bruins, 1975), which is mostly attributed to the slaking of air-dry aggregates by fast rewetting. In our experiment, air-drying was always followed by slow rewetting. Air-drying can result in precipitation of bonding agents at particle-to-particle contact points (Kemper et al., 1987) and an increase in the solid phase cohesion of aggregated particles (Kemper and Rosenau, 1984). With fast wetting, air-pressure builds up inside aggregates, due to the water rapidly entering the soil pores, which will result in a break up of unstable aggregates. Slow wetting on the other hand allows the entrapped air to escape, leading to minimal soil disruption (Kemper and Rosenau, 1984).

After 42 days of incubation, differences between unstable SDW and FDW macroaggregation only occurred in the 2:1 soil. The FDW and SDW treatments differ in two ways. First, SDW soil samples were rewetted when their moisture content reached 50% FC, whereas FDW samples were rewetted after complete air-drying. Less slaking occurs upon rewetting when initial water content of the soil is greater. Second, SDW soils were incubated in open air, and were continuously exposed to drying, whereas FDW soils were incubated at FC during the entire incubation period and only exposed to drying for two days in each FDW cycle. Kemper et al. (1987) reported higher rates of cohesion recovery by aging soil at 0.1 compared to 0.2 kg H_2O kg^{-1} soil, which compares well with the moisture contents at respectively 50% FC and FC in our experiment. This indicates that some tension is needed to pull soil particles into direct contact, which can be established during prolonged air-drying in the SDW treatment. Perfect et al. (1990) have suggested that effects of water content on aggregation depend on factors such as clay mineralogy and soil porosity. The 2:1 clay soil had the largest percentage of sand, which could have caused considerable rapid responses to rewetting and faster water infiltration in the FDW treatment compared to the other two less sandy soils. No differences between the two dry–wet treatments were noticed in these less sandy soils with oxides. This could also indicate that the short drying process in the FDW treatments made the macroaggregates strong enough to resist slaking. This might be attributed to the presence of Fe -and Al-oxides, which are known to have a rather stabilizing effect on aggregation upon drying (Schahabi and Schwertmann, 1970).

Effect of active root growth

Under both FC and SDW conditions, living roots caused a decrease in unstable macroaggregation after 42 days of root growth in all soils. The effects were contrary to our initial hypothesis that root growth would increase aggregation. In the short–term, root growth may stimulate aggregate breakdown and soil fragmentation by creating zones of failure, thereby inducing soil loosening (Materechera et al., 1994). Reid et al. (1982) suggested that decreased stability of aggregates following the short-term growth of corn could be attributed to a destruction of the linkages between organic matter, iron or aluminum, and mineral particles by the roots. In Fe-or Al-rich soils, chelating agents, released in the rhizosphere soil could remove Fe and Al from important linkages. Long-term effects of active root growth, on the other hand, have been associated with biological and physical stabilization of aggregates (Gale et al., 2000; Puget and Drinkwater, 2001; Wander and Yang, 2000). Roots produce cementing agents (root exudates), which can strongly sorb to inorganic materials, thereby helping to stabilize aggregates over time (Tisdall and Oades, 1979). In addition, root exudation stimulates microbial activity with subsequent production of microbial binding agents. Our results suggest that after 42 days of root growth, there was not sufficient production of stabilizing materials to increase stable macroaggregation (Figure 4b), except for the 2:1 soil where a small but significant increase was noticed in stable macro-aggregation with root growth at FC. The effect of root growth on aggregation is largely depending on plant species. Plant species with many fine roots and root hairs are likely to produce more evenly distributed inputs of organic matter that can better stabilize soil

aggregates, compared to coarser and lower-density root systems (Degens, 1997). During sampling, we observed low root densities after 42 days of wheat plant growth.

Roots can also physically influence aggregation by exerting lateral pressures inducing soil compaction, and by localized drying of the soil, which leads to increased sorption and effectiveness of organic binding agents that are released from roots (Czarnes et al., 2000; Reid et al., 1982). In our short-term experiment, drying and wetting forces induced by roots in the SDW (+ plants) treatment may have partially offset the short-term disruptive effects of active root growth, but only in the mixed soil. However, compared to the SDW (−plants) treatment, the aggregate disruptive forces of the roots appeared to be still greater than the stabilizing root-induced drying forces.

Effect of mineralogy

After 42 days incubation of the control treatment (i.e. no added nutrients or organic matter, constant moisture and no plants) aggregation increased in the sequence: 2:1 soil ≤ mixed soil < 1:1 soil (Figure 1a). Because microbial activity was similar across the three soils (data not shown), we did not attribute these differences in aggregation to initial differences in nutrient status or OC concentrations between soils. Soil physical properties and electrostatic interactions are believed to play an important role in aggregate formation. In soils dominated by 2:1 clay minerals, negative surface charges of SOM and negative charges in the body of clay minerals are mutually bound with positively charged polyvalent metal cations (Edwards and Bremner, 1967). Highly weathered soils of the tropics are generally well known for their large content of variably-charged mineral particles (oxides and 1:1 clay minerals). The potential of oxides to form micro- and macroaggregates is well known and thought to be a result of electrostatic interactions between positive charges associated with oxides and negative charges of crystalline clay minerals (Kemper and Koch, 1966; Pinheiro-Dick and Schwertmann, 1996). It has also been reported that 1:1 minerals have a high flocculation capacity caused by electrostatic interactions between positive and negative charges respectively on the edges and in the body of the clay mineral. Both charges co-exist at prevailing field pH (El-Swaify, 1980). Zero points of charge (ZPC) for 1:1 clay minerals have been reported from < 3.5 to 4.6 (Parks, 1965) up to as high as 8–10 (Schofield and Samson, 1954).

The co-existence of negative and positive charges at prevailing field pH can explain the capacity of these soils for mineral-mineral binding (Oades and Waters, 1991; Six et al., in press). Moreover, in the presence of oxides, this mineral binding tendency is enhanced because of higher ZPC values of both Fe(III)-and Al-oxides and their hydroxides (El-Swaify, 1980; Parks, 1965) compared to kaolinites. Due to the greater potential of soils dominated by 1:1 clay minerals and Fe- and Al-oxides to form aggregates by mineral–mineral bindings, SOM may be less important as a binding agent in stabilizing aggregates in these soils compared to soils dominated by 2:1 clay minerals (Six et al., 2000a; Tisdall and Oades, 1982).

Addition of SOM in our experiment caused significant increases in macroaggregation in all three soils. Interestingly, the relative increases in macroaggregation significantly differed between the three soils, though increases in microbial activity were similar for all soils. The lowest increase in unstable and stable macroaggregation was noticed in the 1:1 soil, whereas macroaggregation in the 2:1 and mixed soils was largely affected by OC input. Moreover, in the treatment without nutrients and with low OC inputs, stable macroaggregation decreased after day 14 in soils with 2:1 clay minerals, whereas no changes in macroaggregation were noticed over time for the soil dominated by 1:1 clay minerals. Readily available OC becomes depleted over time due to microbial decomposition, and subsequently microbial activity and production of binding agents decreases. In soils where SOM is the major binding agent for aggregates, this results in a destabilization of the macroaggregates (Six et al., 1998). In soils where mineral surfaces are directly bound by electrostatic interactions, depletion of SOM may have less impact on aggregate stability. Also, stable macroaggregation was significantly enhanced by root growth in the 2:1 soil only (Figure 4b), indicating that roots can have an aggregate stabilizing effect in soils where SOM plays a major role in aggregate stabilization.

It has been shown that soil clay content can influence the degree of aggregation (Franzleubbers and Arshad, 1996; Kemper and Koch, 1966) because the higher reactive surface of clay promotes binding with organic materials. A positive relationship between SOM stabilization and clay content has been demonstrated (Hassink, 1997; Merckx et al., 1985). However, much less is known about the interaction between texture and mineralogy as it affects aggregate formation and SOM stabilization. The soils used in
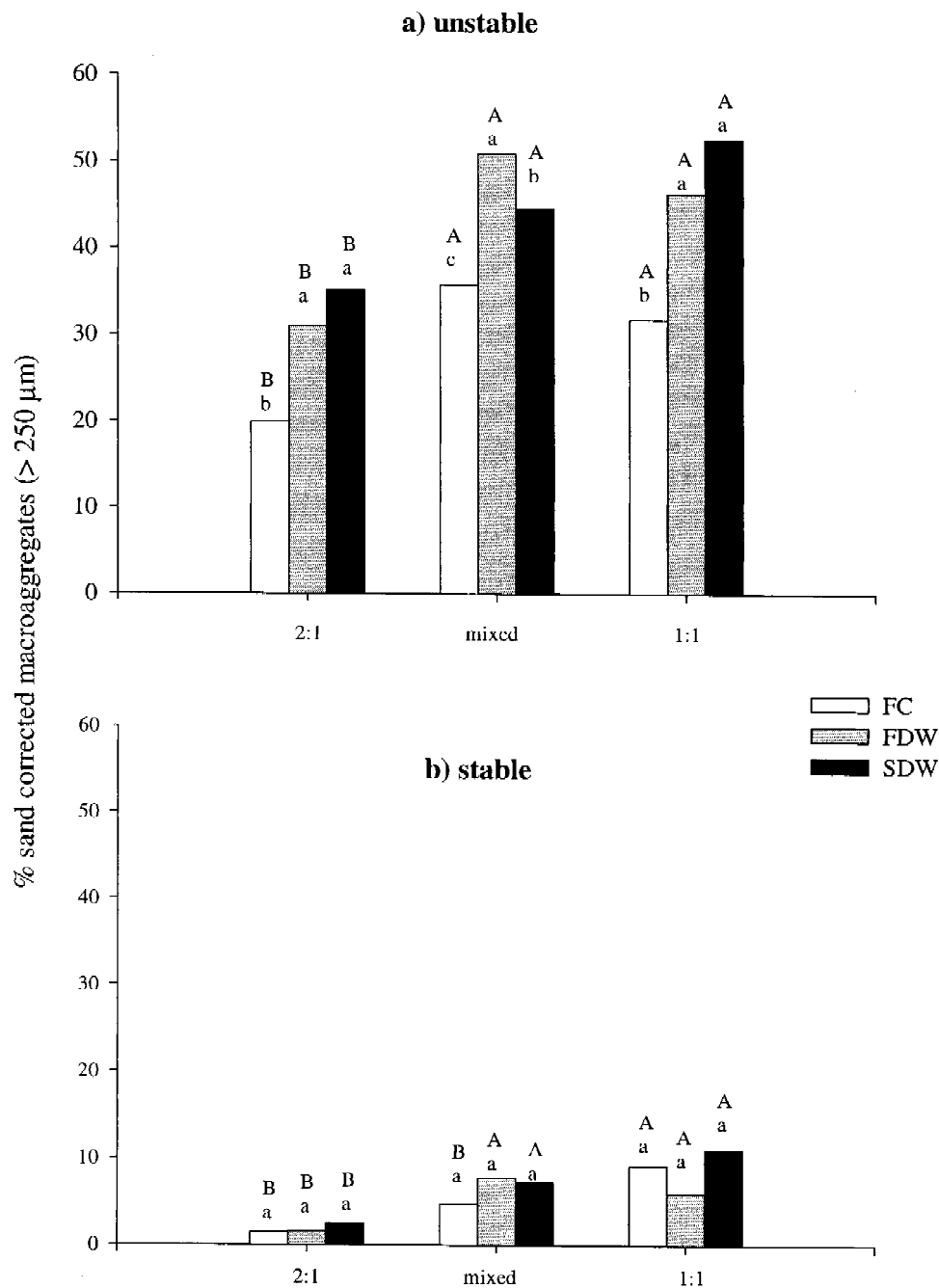
Figure 3. Effect of dry–wet cycles on unstable (a) and stable (b) macroaggregation (% sand corrected macroaggregates >250 μm) after 14 days of incubation with low input of OC (0.3 g wheat straw 100 g^{-1} soil). FC = maintained at field capacity; FDW = fast air-drying and slow rewetting; SDW = slow air-drying (up to 50% field capacity) and slow rewetting. Axis labels as in Figure 1. Values followed by a different uppercase letter within the same treatment/stability and among soils are significantly different ($P < 0.05$). Values followed by a different lowercase letter within the same soil/stability and among treatments are significantly different ($P < 0.05$).
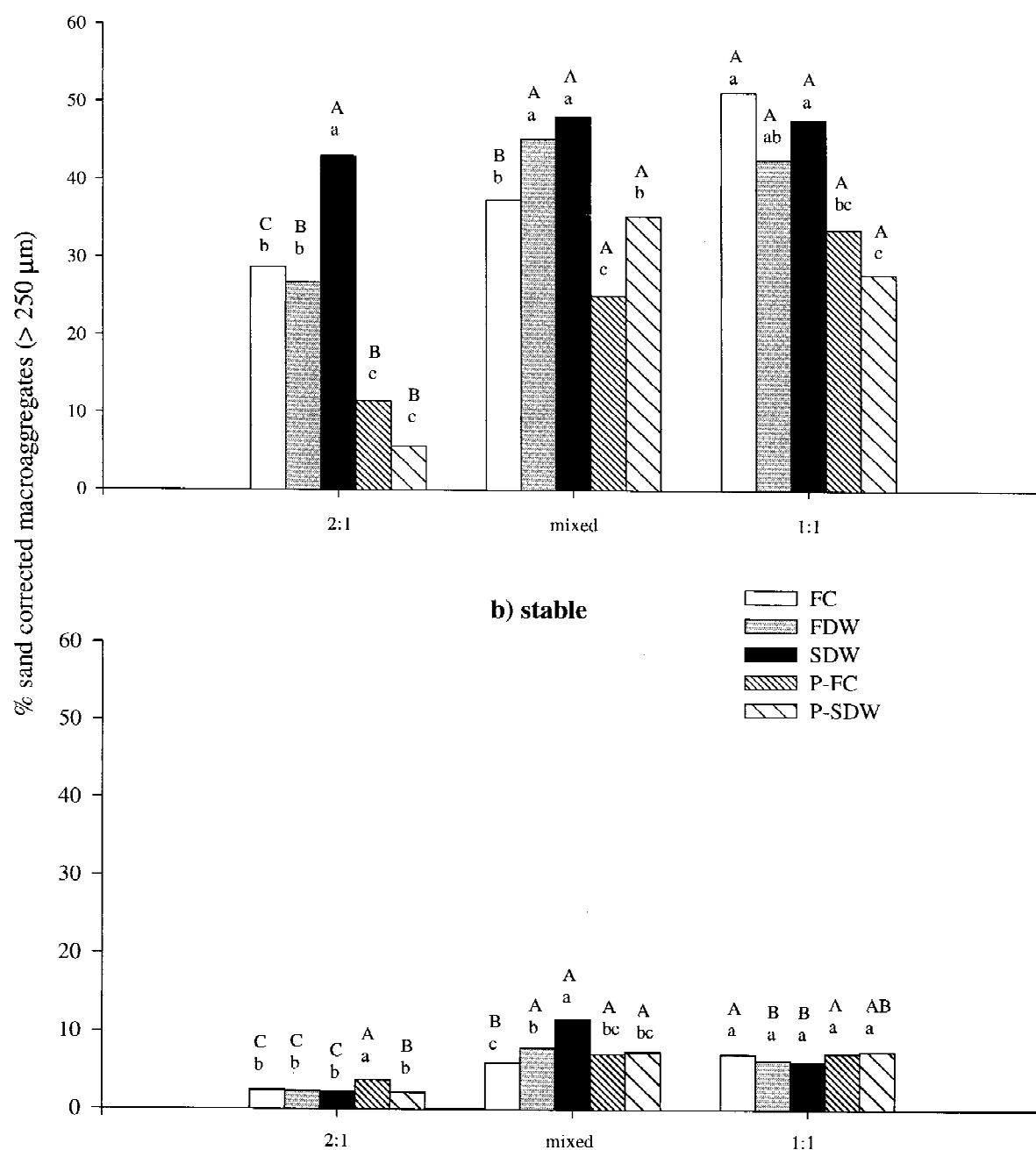
Figure 4. Effect of dry–wet cycles and plant-and root growth on unstable (a) and stable (b) macroaggregation (% sand corrected macroaggregates >250 μm) after 42 days of incubation with low input of OC (0.3 g wheat straw 100 g^{-1} soil). FC = maintained at field capacity; FDW = fast air-drying and slow rewetting; SDW = slow air-drying (up to 50% field capacity) and slow rewetting; P = with wheat plants. Axis labels as in Figure 1. Values followed by a different uppercase letter within the same treatment/stability and among soils are significantly different ($P < 0.05$). Values followed by a different lowercase letter within the same soil/stability and among treatments are significantly different ($P < 0.05$).

this study differed substantially in both clay content and mineralogy. Interestingly, in the low OC treatment, aggregation increased with increasing amount of 1:1 clay minerals and oxides, but not with total clay content. With high OC inputs, the mixed soil, which has the lowest total clay content but the highest CEC due to the presence of vermiculites (Alexiades and Jackson, 1965), showed the highest amount of macroaggregation and the greatest response in macroaggregation to OC input. This indicates that mineralogy and charge density can be more important parameters for aggregate formation and stabilization than total clay content.

Comparing unstable versus stable macroaggregation in all soils, macroaggregates formed in the mixed soil seem to be the least susceptible to the disruptive effect of slaking compared the other two soils. This indicates that in soils with a mixture of 2:1 clays, 1:1 clays and oxides, drying prior to wet–sieving (in the slaking treatment) has a higher stabilizing capacity which overshadowed the disruptive effect of rewetting compared to soils dominated by only 2:1 or 1:1 clay minerals. Schahabi and Schwertmann (1970) also showed that drying was essential for the stabilization of aggregates in soils where suspensions of iron-polycations were added. They suggested that the fine iron oxides are sorbed in excess of the cation exchange capacity on the external faces of the clay domains and then migrate on drying. However, in the 1:1 soil, macroaggregates were more disrupted by slaking than in the mixed soil, but only in the high OC treatment. In the low OC treatment, both soils decreased 76% to 86% in macroaggregation upon slaking at respectively days 14 and 42 (Figure 2a). We suggest that the presence of vermiculites in the mixed soil resulted in strong interactions between the 2:1 clay minerals and the organic matter in the high OC treatment. Due to hydrophobic characteristics of SOM (Caron et al., 1992) and the formation of additional intermolecular associations upon drying, aggregates in the vermiculite soil may have reduced wettability compared to the 1:1 clay soil, resulting in less disruption of macroaggregates upon slaking.

Compared to the control soil, unstable macroaggregation at day 42 was positively affected by frequent SDW in the two soils containing 2:1 clay minerals, but not in the 1:1 soil. Drying is believed to increase surface acidity (Sposito, 1989), which will result in more positive charges and a subsequent greater specific adsorption of organic molecules onto 2:1 clay mineral surfaces (which have permanent negative charges).

However, 1:1 soils have variable charged minerals that generally carry positive charges at field pH. When exposed to drying, these positively charged minerals may have less potential to bind with SOM.

Conclusions

Short-term soil aggregate formation and stabilization was considerably affected by soil clay mineralogy. Under low OC levels, highly weathered soils (dominated by 1:1 clay minerals and oxides) seemed to have the largest capacity to form macroaggregates. Although SOM had a significant positive effect in all soils on the formation of water-stable macroaggregates, the macroaggregate formation increase upon SOM addition was the smallest in soils dominated by 1:1 clay minerals and oxides. Therefore, we conclude that macroaggregate formation is less dependent on SOM in this type of soil compared to mixed and 2:1 mineral dominated soils. Nutrient addition seemed to alter soil physical properties rather than microbial activity, which resulted in decreased versus increased aggregation in the mixed soil versus the 2:1 and 1:1 soils. Drying and wetting caused a fast increase in unstable macroaggregate formation in all three soils but had no effect on stable macroaggregation. Drying seemed to have a more stabilizing effect in the soils with oxides compared to the 2:1 soil. Active root growth of wheat plants caused disruption of unstable aggregates in all three soils. Stable macroaggregation was significantly enhanced by root growth in the 2:1 soil only, indicating that roots can have an aggregate stabilizing effect in soils where SOM plays a major role in aggregate stabilization. When OC was not limiting, the presence and subsequent interactions of 2:1 and 1:1 clay minerals, oxides and organic matter compounds were suggested to cause the highest levels of stable macroaggregation.

Acknowledgements

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