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Carbonatite rock can enhance plant growth and nutrition depending on crop traits

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Abstract

Aims The increasing global demand for sustainably-produced crops has led to a renewed interest in exploiting unprocessed rocks as soil amendments and fertilizers. Carbonatite rocks are of particular relevance because of their rapid weathering rates and diverse nutrient contents. However, there are insufficient data to support or refute their efficacy and to understand their mechanism(s) of action. Here, the effects of a carbonatite on two crops were assessed and compared to those of calcitic lime.

Methods Wheat and pea were repeatedly grown under a low-nutrient regime under greenhouse conditions and their development, biomass, and shoot nutrient content were measured. The effect of the carbonatite on soil CO_2 evolution was also tested for wheat.

Results Wheat grown with carbonatite produced 40% more shoot biomass and 50% more root biomass than plants grown with lime. There was a sharp reduction in specific root length (SRL), consistent with approximately 60% increases in shoot contents of N, P, K, and Mn. These effects were smaller for pea. For wheat, CO_2 from the soil was 70% greater with lime than with carbonatite.

Conclusions We conclude that carbonatites can provide benefits to plants beyond serving as liming agents. In addition, root architecture and SRL appear to be useful traits for predicting plant responsiveness to carbonatite addition.

Keywords Rock fertilizers · Carbonatite · Specific-root length · *Pisum sativum* · *Triticum aestivum* · Sustainable agriculture

Abbreviations

DAP	Days after planting
DW	Dry weight
HSD	Honest Significant Difference
R:S ratio	Root:shoot ratio
SRL	Specific root length
SRC	Spanish River Carbonatite

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Introduction

One of the biggest challenges of modern agriculture is to ensure long-term productivity while maintaining the stability of agroecosystems (Conway 1987; Gliessman 2004). Given the broad diversity of crops and growing conditions in agriculture, it is clear that no single tool or approach will achieve sustainability, and that tailored solutions for specific agroecosystems are urgently needed. In this context, the application of new sustainable techniques or tools is paramount. Using minimally-processed rocks as amendments for cropping systems is one approach that has shown potential in recent years (e.g., Myrvang et al. 2017; Zhang et al. 2018). However, a major limitation of their use results from the insoluble nature of most rock types. Silicate rocks, for example, often do not deliver sufficient nutrients to justify their application as rock fertilizers (Harley and Gilkes 2000). Carbonatite rocks differ from silicate rocks because they have relatively high weathering rates. They are predominantly composed of carbonate-minerals such as calcite (Woolley and Kempe 1989) and can contain one or more of 29 nutrient-bearing minerals potentially useful for agriculture (Jones et al. 2020). Several reports have suggested that they can be effective nutrient sources for plants with nutrient delivery rates similar to those of chemical fertilizers (Bakken et al. 1997a, b; Myrvang et al. 2017) and can be used as liming materials (e.g., Myrvang et al. 2017). Despite this, many questions remain as to their effects on plants and whether other agroecosystem components, like soil microorganisms, are affected because of their use. Answering these questions is complicated by the diverse composition of these rocks, which can lead to them being viewed as a lime, a rock fertilizer, or both. Thus, it is essential to further our knowledge on carbonatite rocks in order to predict outcomes and to develop best practices for their use in agriculture.

In a study of the naturally-occurring plant and soil microbial communities present at a carbonatite deposit in northern Ontario, Canada, Jones et al. (2019) noted that both of these communities were affected by the presence of the carbonatite. In areas under carbonatite influence, the plant communities shifted to include more disturbance-tolerant species, but the bacterial and fungal communities had a more nuanced response. Only OTUs representative of the Micrococcaceae and Gaiellaceae families were found to increase with carbonatite influence; otherwise, bacterial communities were mostly unchanged. Carbonatite influence was also associated with a decline in fungal diversity in a manner consistent with a pH-based effect (Jones et al. 2019). These data raise questions about plant-carbonatite-soil microbe interactions in agricultural systems. Soil type, plant traits, and microorganisms are likely to be crucial determinants in whether carbonatites are effective amendments. For instance, carbonatites will probably not benefit crops when used in soils of high pH and/or rich in calcium, as these soil characteristics are not conducive to the weathering of minerals (Arcand et al. 2010; Jones et al. 2020). As well, carbonatites may not benefit all plants to the same extent because different crops display different nutrient acquisition strategies which likely affect element release and nutrient uptake from rocks. Some plants have thin roots that can access a high volume of soil whereas others possess thick roots that intensely mine a small volume of soil (Wen et al. 2019). Finally, the rate of carbonatite mineral weathering will likely depend on the soil microorganisms present as these are known to play a key role in the release of mineral-bound elements as shown in geochemical tests (Rogers and Bennett 2004), forest soils (Uroz et al. 2007, 2009, 2011), the rhizosphere (Whitman et al. 2018), and agricultural soils (Basak and Biswas 2009).

In this study, we used a representative type of agricultural carbonatite found in Canada which has been commercialized under the name SRC or Spanish River Carbonatite. Its three main minerals are calcite, apatite, and biotite (Sage 1987), which could provide Ca, P, Mg, and K to soil systems. Sedimentary calcite is a common liming agent (Holland et al. 2018), and the igneous calcite present in SRC is expected to have similar effects on agroecosystems. When compared to the Lillebukt Alkaline Complex Carbonatite from Stjernøy, Norway, which has been used in several agricultural studies (Bakken et al. 1997a, b; Myrvang et al. 2016, 2017), SRC exhibits low levels of Ba. Furthermore, it lacks other potentially harmful elements found in some carbonatite deposits (e.g., U or Pb; Heinrich 1980; see Sage, 1987, for SRC mineralogy and geochemistry). Therefore, SRC represents an ideal carbonatite to test predictions on the agricultural

use of these rocks. Based on its mineralogical composition and the positive agricultural effects seen with other carbonatites (Jones et al. 2020), it is expected that SRC would benefit crops and agroecosystems as a whole by increasing soil pH, influencing soil geochemistry, and acting as a nutrient source. However, the magnitude of the effect(s) and the factors driving such benefits remain to be determined.

Here, we assess the effects of the carbonatite SRC on wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L). Our objectives are twofold: 1) to compare SRC to calcitic lime in terms of their effects on plant growth and nutrition when both amendments are used equally to adjust the soil pH, and 2) to determine if wheat and pea, two globally important crops, differ in their response to carbonatite amendment.

Materials and methods

Experimental design

Two experiments were conducted: one with wheat cv. Norwell and one with pea cv. Sparkle. A 1:1 (vol:vol) mixture of vermiculite:Turface® (Table 1) was used as a common low-nutrient substrate in all experiments to minimize the influence of soil nutrients and organic matter and to replicate conditions under which rock weathering should occur (i.e., a nutrient-depleted and acidic soil; Jones et al. 2020). The SRC has been mined for several years by Boreal Agrominerals Inc. who sells the crushed rock as an agricultural amendment similar to lime; the company recommends using a ratio of 1:10 (vol:vol) SRC:substrate for horticultural applications. We chose to use this ratio here as preliminary work had confirmed that, when used in pot-based experiments, it was optimal for pea growth and for stable soil pH over time (Jones 2016). Calcitic lime was used in a ratio of 1:15 (vol:vol) lime:substrate to match the pH obtained with SRC at the time of planting. The grain size and some properties of the substrates and amendments is provided in Table 1, and the SRC nutrient content and particle size distribution are given in Table S1. The carbonatite or lime was homogenously mixed with the substrate and deionized water was added to give a 20% (vol/vol) water content. The amended substrates used for pea were autoclaved at 121 °C and 33 psi for 2 h prior to planting to avoid introduction of microorganisms from either the substrates or the amendments. With wheat, the experiments required large substrate volumes so it was not feasible to autoclave them; thus, only the amendments were autoclaved to remove any amendment-specific microorganisms.

For wheat, a full life-cycle study was conducted with the assessment of weekly growth and final biomass production. Wheat seeds were randomly assigned to one of three substrates: substrates amended with calcitic lime, SRC, or silica sand. The silica sand, which was added to the substrate at a ratio of 1:10 (vol:vol), was used to control for changes in substrate texture, and to verify which effects resulted from substrate pH changes. A total of fifteen plants were grown per treatment, and five plants from each one were left to grow until senescence for yield determination. The experiment was repeated twice, with trial one grown from Nov 22/2017 to Jan 17/2018, and trial two grown from Mar 18/2018 to May 13/2018. Seedlings were inoculated with 10 mL of an agricultural microbial mixture immediately after planting (see below for preparation). Although two replicate trials were conducted, each was analyzed separately due to seasonal environmental differences within the greenhouse (Table S2). Because the second trial took place under growing conditions considered optimal for wheat (e.g., longer photoperiods; http://www.fao.org/land-water/databases-and-software/crop-information/wheat/en/), this trial is presented as

Table 1 Properties of the common substrate components and amendments used during experiments

Mineral	Primary elements	Grain size (mm)	Source
Vermiculite	Mg, Al, Fe, Si	1–6	Plant Products Co. (ON, CA)
Turface®	Al, Fe, Si	0.8—3.4	Plant Products Co. (ON, CA)
Silica Sand	Si	~1	Bell & MacKenzie Co Ltd. (ON, CA)
SRC	Ca, K, Mg, P	0.3—2.0	Boreal Agrominerals Inc. (ON, CA)
Calcitic Lime	Ca	2.5	Quality Fertilizers, Inc. (ON, CA)

representative and differences between the trials are noted where applicable. Data from the first trial are available as supplementary data.

For pea, preliminary work had suggested an interaction between carbonatite, pea, and root symbionts (Jones 2016), and so further emphasis was placed on the microbial component. Seeds were randomly assigned to one of four treatment groups. The control substrates were amended with lime (LC) or carbonatite (SC; 1:15 or 1:10 vol:vol, respectively), and two treatment groups were inoculated with an agricultural microorganism solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes; see below for preparation). For each treatment group, eight seeds were assigned per experimental replicate, and the experiment was replicated twice.

A microbial inoculant was used to provide an agriculturally-relevant soil microbial community. The microbial solution was prepared from a variety of agricultural soils, collected in Ontario, Canada (Table S3), which were stored at 4 °C until used. These soils were pooled to minimize microorganism effects from any single source, and microorganisms were extracted by shaking a 1:9 (vol:vol) soil:deionized water mixture at 125 rpm for 2 h, discarding the soil, and retaining the liquid as an inoculant (adapted from Lindahl and Bakken 1995). The solution was made fresh (< 24 h before use) for each experiment and was stored at 4 °C prior to use.

Experiment 1: *Triticum aestivum* growth responses to carbonatite

Untreated seeds of wheat (Cribit seeds; West Montrose, ON, Canada) were surface-sterilized (Sauer and Burroughs 1986) and pre-germinated for 48 h (Wu et al. 2007). Three germinated seeds were planted per 3.78 L pot at a depth of 1 cm, and thinned within 1 day of establishment to keep the largest emerged seedling. Plants were grown in the greenhouse at the Cold Regions and Water Science Building at Wilfrid Laurier University (WLU) in Waterloo, Ontario, Canada. Average photoperiods, temperatures, and ambient humidity for the growing periods can be found in Table S2. Plants were watered individually from above every 2–4 days with deionized water, and after 14 days of growth, they were given chemical fertilizer (Miracle-Gro® commercial fertilizer at 2 g/L, N:P:K of 24:8:16) every third watering (see Table S4 for a detailed composition of the fertilizer).

Each week following planting, plant growth was assessed by recording the Zadoks' developmental stage (Zadoks et al. 1974). The length of the longest leaf was also measured, from the soil surface to its tip. Plants were harvested at 56 days after planting (DAP), time which corresponded to the peak vegetative growth under our conditions. At this time, plants were removed from the soil, the shoot (comprising stem, leaves, and tillers) was separated from the roots, and the roots cleaned of all substrate particles. Two root characteristics were then determined to examine whether amendments were specifically altering root architecture: the overall root length and the total root surface area. These parameters were obtained using the Arabidopsis WinRhizo™ software and EPSON 10000XL 3.49 Flatbed scanner system (Regent Instruments Inc., Canada; software version 2012d). Following drying for at least 72 h at 60 °C, the dry weights (DW) of the shoots and roots were taken. The concentration of macro-nutrients (N, P, K, Ca, Mg, S) and micro-nutrients (Fe, Al, Mn, B, Cu, Zn) in dried shoots was determined by acid-digestion and inductively-coupled plasma spectroscopy carried out independently by Actlabs Agriculture Laboratories (Ancaster, ON, Canada). To obtain nutrient content, shoot DW was multiplied by the ppm concentrations for each element. To gain insight into the effect of SRC and lime on the release of CO_2 from soils (i.e., through soil microbial activity or carbonate breakdown), the soil CO_2 evolution was measured by alkali trap (Rowell 1995) using Magenta™ jars. The soil pH was measured on a 1:1 (vol:vol) mixture of deionized water:soil (Watson and Brown 1998). For yield measurements, seeds were collected at senescence from the five plants per treatment which had been left to grow and the number and total DW of seeds were determined on a per-plant basis.

To gain further insight into how the amendments might be affecting plant growth, three other parameters were calculated. First, the root to shoot (R:S) biomass ratio was calculated as an indicator of biomass investment responses to substrate nutrients (Gruber et al. 2013). Second, the specific root length (SRL; m g^{-1} root DW) for each plant was determined by dividing the total root length by the total root DW in order to assess the plant's nutrient foraging strategy (Freschet and Roumet 2017; Wen et al. 2019). Finally,

growth rates for wheat (cm per week) were calculated by using the measured weekly length of the longest leaf in a linear regression (the "lm" function; R software suite version 3.5.2) to obtain the growth rate of each individual plant over time.

Experiment 2: microbial interaction study with *Pisum sativum*

Pea seeds, obtained from a genetically-stable line (cv. Sparkle deposited at the John Innes Centre, Norwich, UK, and propagated at WLU), were surface-sterilized and imbibed overnight (Guinel and Sloetjes 2000). They were then individually planted in black Cone-tainers™ (656 mL volume; Stuewe and Sons, Tangent, OR, USA) which were placed in trays filled with 1.5 L of deionized water; the trays were separated by treatment group. Seedlings were watered by adding 1.5 L deionized water to the tray when needed, and after 10 days, plants were provided every third watering with a modified Hoagland solution (Guinel and Sloetjes 2000); the chemical composition of which is provided in Table S4. Plants were grown in the growth-room facility at WLU under a 16-h day (23 °C) and 8-h night (18 °C) photoperiod cycle. Light was supplied through high-pressure sodium, fluorescent, and metal halide bulbs, which provided 250 µmol m^{-2} s^{-1} of photosynthetically-active radiation (measured with a LI250A LICOR Biosciences light meter, Lincoln, NE, USA).

Plants were harvested 21 DAP, i.e., during vegetative growth. The shoot and root biomass, the soil pH, the root length and surface area, and the shoot nutrient contents were determined as described above. The R:S biomass ratio and the SRL were also calculated. Additionally, as inoculated plants produced root nodules, the number and DW of those nodules were determined per plant.

Statistical analyses

All statistical analyses were completed using the R software suite (version 3.5.2; http://www.r-project.org/). For wheat, each trial was analysed separately due to seasonal differences in growing conditions. Because parameters for wheat were often not normally distributed, the mean measurements between treatments were compared using a Kruskal–Wallis test followed by a post-hoc Dunn's test using the 'dunntest' function included in the FSA package (Ogle et al. 2019). For pea, the growing conditions were controlled between trials and considered identical. Therefore, trials were analyzed together if no significant trial effect was observed. Differences in each parameter between treatments were tested using a one-way ANOVA. When treatments were shown to be a significant source of variation (≥ 95% confidence level), a Tukey Honest Significant Difference (HSD) post-hoc test was used to identify treatment-specific differences. Normality was tested using the Shapiro-Wilks test on residuals extracted from each ANOVA model. Finally, differences between lime and carbonatite treatments for pea were further clarified by pooling the treatments by amendment regardless of inoculation. The statistical tests previously used to analyse the pea parameters were re-run with these two new treatment groups (lime, i.e., LC and LAG together, and SRC, i.e., SC and SAG together).

Results

Wheat growth responses to carbonatite

Wheat responded strongly and positively to SRC, and the plant responses across the two trials were consistent, although they differed in magnitude. Because the plants in trial two were grown under optimal seasonal conditions for wheat, we chose this trial as representative. Stark differences between the two trials are indicated in the text when present and results from trial one are included as supplemental data (Table S5 and Figures S1, S2). Plants grown with carbonatite had, as early as two weeks after planting, longer leaves than those in the other treatments (Fig. 1a). Furthermore, the average growth rate for plants in SRC-amended substrates was significantly higher (9.2 ± 0.1 cm $week^{-1}$) than that of control plants (8.3 ± 0.2 cm $week^{-1}$) or plants grown in lime-amended substrates (8.2 ± 0.3 cm $week^{-1}$). This was also observed, albeit to a greater extent, in the first trial where the longest leaf of plants grown in SRC-amended substrates grew about 3.5 cm more per week (8.9 ± 0.2 cm $week^{-1}$) than that of control plants (5.5 ± 0.2 cm $week^{-1}$) or of plants in lime-amended substrate (5.1 ± 0.2 cm $week^{-1}$). At 56 DAP, the shoot biomass of carbonatite-treated plants was

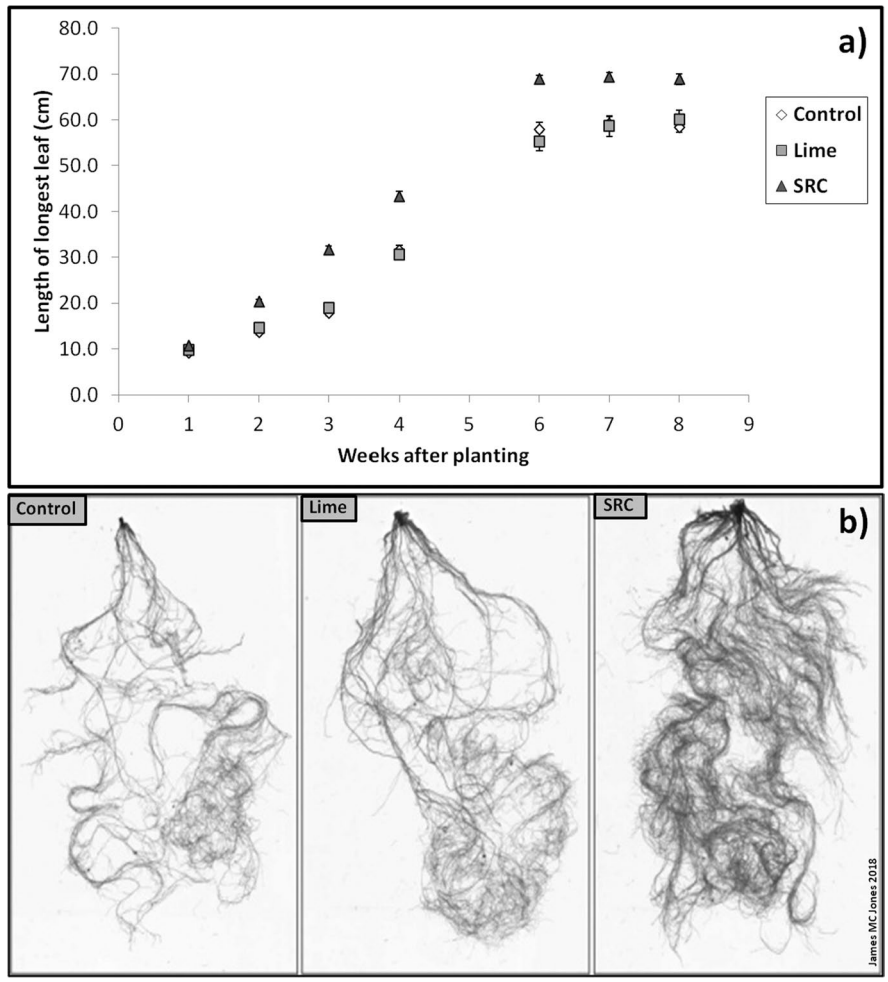

Fig. 1 **a** Length of the longest leaf of wheat plants (mean ± standard error) grown for eight weeks; the data presented are for trial two, which we considered representative of the two trials performed. Plants ($n = 14$–15 per treatment) were grown in substrates with either 1:10 silica sand:substrate (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC). Data were not obtained for week 5 because of a local ice storm which prevented taking measurements. **b** Representative scans of 56 day-old wheat plant root systems from trial two. The scanning areas are 40 cm in length

nearly 40% higher than that of lime-treated or control plants (Fig. 2a). Additionally, at that age, their root biomass was 50% greater than that of plants grown in the other two treatments (Fig. 2a). This large increase in biomass in carbonatite-treated plants reflected a higher number of lateral roots, a higher density of roots per unit volume, and a greater number of nodal roots (Fig. 1b). Total root length and total root surface area were the highest in plants grown with carbonatite (Table 2). Consequently, the SRL was significantly lower in these plants than in plants grown in the other treatments (Table 2). Similar trends were observed in trial one for biomass (Figure S1) and root architecture parameters (Table S5). It should be noted that no mycorrhizal fungal colonization of roots was observed in either trial.

After eight weeks of plant growth, lime- and carbonatite-treated substrates both had a significantly higher pH (7.66 ± 0.05 and 7.27 ± 0.03, respectively) than that of the silica sand control (5.89 ± 0.03). Plants developed at similar rates in all cases, and no differences in Zadoks' developmental stages between treatments or trials were seen. Two parameters which differed strongly between the two trials were soil respiration and yield. In trial one, soil CO_2 evolution was similar for all substrates (~ 1.25 ± 0.01 ng CO_2 g^{-1} soil DW s^{-1}), whereas in trial two it was significantly greater in the lime treatment (0.10 ± 0.01 ng CO_2 g^{-1} DW s^{-1}) than in both carbonatite-amended and control treatments (0.03 ± 0.01 ng CO_2 g^{-1} DW s^{-1}). As for yield, in trial two, plants produced similar numbers of seeds which were also of similar weight. Plants grown with sand produced an average of 70 ± 5 seeds, with lime an average of 89 ± 5 seeds, and with carbonatite an average of 67 ± 5 seeds. The average total seed DW per plant was 2.43 ± 0.16 g for plants

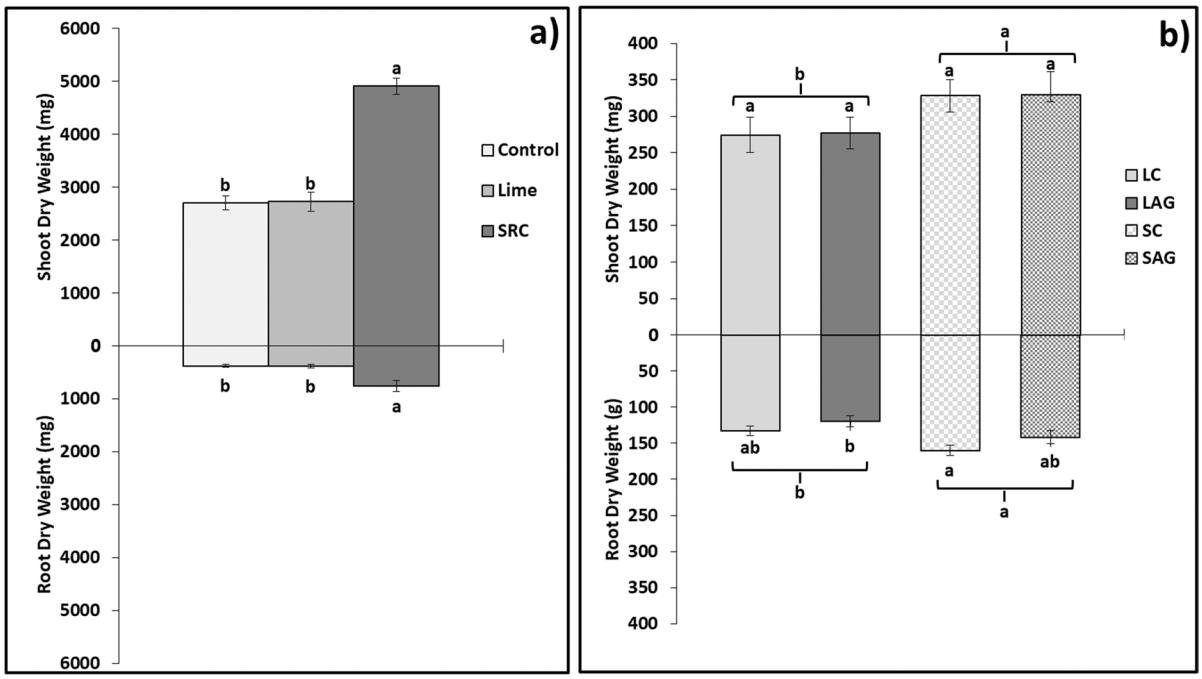

Fig. 2 **a** Shoot and root biomass of 56 day-old wheat plants (n = 10 per treatment) from trial two, which we considered representative of the two trials performed. Plants were grown in substrates with either silica sand:substrate (control), calcitic lime:substrate (lime), or SRC:substrate (SRC). Significant differences in biomass are indicated by different letters (Kruskal–Wallis + post-hoc Dunn's test at 95% confidence level). **b** Shoot and root biomass of 21 day-old pea ($n = 14$–15 per treatment) grown either in lime:substrate (LC) or SRC:substrate (SC), or in the same substrates but inoculated with an agricultural microbial solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes). Significant differences between treatment groups are indicated by different letters (One-way ANOVA at 95% confidence level). For both sets of plants, values presented are means ± standard error. When treatments were pooled by amendment, differences between plants are shown as different letters outside of the brackets (One-way ANOVA at 95% confidence level)

grown with sand, 2.89 ± 0.13 g for plants grown with lime, and 2.39 ± 0.18 g for plants grown with the carbonatite. In contrast, in trial one, carbonatite-treated plants produced significantly more seeds per plant (140.2 ± 13.0) than lime-treated (86.0 ± 8.3) or control plants (92.5 ± 9.8). The total seed weight of carbonatite-treated plants was also significantly heavier per plant (4.99 ± 0.52 g) than that of lime-treated plants (2.80 ± 0.32 g), and that of control plants was intermediate in value (3.39 ± 0.31 g).

Several macro-nutrients, i.e., N, P, and K, were present in significantly higher quantities in the

Table 2 Root architecture parameters of 56 day-old wheat from trial two, which we considered representative of the two trials performed. Plants were grown in substrates amended with either 1:10 silica sand:substrate (control), 1:15 calcitic lime:substrate (lime), or 1:10 SRC:substrate (SRC)

	Control	Lime	SRC
Total RL (cm)	3683.02 ± 199.91 b	4085.41 ± 204.63 b	4906.35 ± 168.80 a
Total RSA (cm^2)	133.75 ± 9.88 b	128.21 ± 8.95 b	187.41 ± 7.13 a
SRL (m g^{-1} root DW)	100.37 ± 4.80 a	110.35 ± 5.87 a	61.37 ± 5.63 b

Values are presented as means ± standard error ($n = 10$ per treatment). Significant differences are indicated by different letters (Kruskal–Wallis + post-hoc Dunn's test at 95% confidence level)

RL root length, *RSA* root surface area, *SRL* specific root length, *DW* dry weight

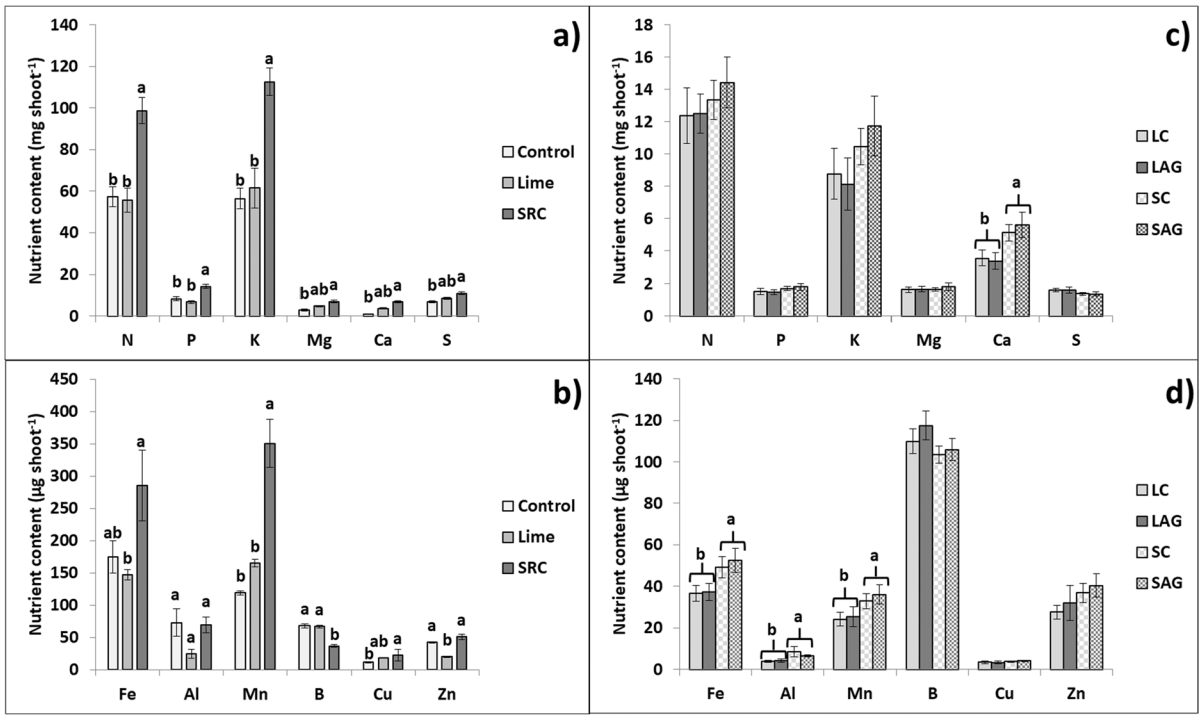

Fig. 3 Average shoot macro- **(a)** and micro- nutrient **(b)** content ± standard error of 56 day-old wheat from trial two. Plants were grown in substrates amended with either lime:substrate, SRC:substrate, or silica sand:substrate (control). Significant differences between treatment groups for each element are indicated by different letters (Kruskal–Wallis and Dunn's tests at 95% confidence). $n = 5$–6 samples per treatment per trial. Average macro- **(c)** and micro- nutrient **(d)** content ± standard error of 21 day-old pea shoots. Plants were grown in substrates with either lime (L) or SRC (S) and inoculated or not with an agricultural microbial solution (AG or C, respectively). Within each treatment, the dried shoots were pooled before analysis to give 6 samples per treatment group across the two trials. No significant differences between treatment groups were detected (One-way ANOVA and Tukey HSD tests at 95% confidence) when treatments were considered individually. When treatments were pooled by amendment, differences are shown as different letters outside of the brackets (One-way ANOVA at 95% confidence level)

shoot of carbonatite-treated plants than in shoots of plants grown in the other treatments (Fig. 3a), while others such as Mg, Ca, and S were found in similar amounts in lime- and carbonatite-treated plants. There was no obvious pattern for the shoot micronutrients. However, the B levels were notably lower in carbonatite-treated plants than in plants grown in the other treatments (Fig. 3b), and the levels of Zn were the smallest in plants grown in lime-amended substrates (Fig. 3b). Manganese was the only micronutrient which significantly increased in carbonatite-treated plants compared to those grown in the other treatments: in plants grown with carbonatite, Mn content was twice as high as in plants grown with lime or with the sand controls (Fig. 3b). Trends similar to these were observed in trial one (Figure S2).

Pea growth responses to carbonatite and interactions with microorganisms

No differences were observed across treatment groups in the shoot biomass of pea plants 21 DAP (Fig. 2b). There were also no observed differences in root biomass or root:shoot ratios among treatments (Fig. 2b; Table 3). Although the root parameters were largely unchanged between treatments, there were two key differences. The first was in the total root length, where root systems of inoculated plants grown with carbonatite were significantly longer than those of inoculated plants grown with lime (Table 3). The second was a difference in total root surface area, whereby roots of carbonatite-treated plants, inoculated or not, had a significantly larger surface area than those of inoculated

Table 3 Root: shoot (R:S) biomass ratio, and root architecture parameters of 21 day-old pea grown either in control substrates amended with lime (LC) or SRC (SC), or in similar substrates inoculated with an agricultural microbial solution (LAG for lime with agricultural microbes and SAG for SRC with agricultural microbes)

	LC	LAG	SC	SAG
R:S biomass ratio	0. 51 ± 0.03 a	0.44 ± 0.02 a	0.50 ± 0.02 a	0.46 ± 0.03 a
Total RL (cm)	1163.26 ± 70.00 ab	1006.09 ± 46.67 b	1190.78 ± 46.04 ab	1207.52 ± 60.66 a
Total RSA (cm^2)	228.58 ± 12.99 ab	202.28 ± 10.82 b	251.43 ± 11.11 a	247.88 ± 15.38 a
SRL ($m\ g^{-1}$)	87.82 ± 2.39 a	85.67 ± 2.19 a	74.78 ± 1.28 b	87.23 ± 2.55 a

Values are presented as means ± standard error ($n = 14$–15 per treatment). Significant differences between treatment groups for each parameter are indicated by different letters (One-way ANOVA at 95% confidence level)

RL root length, *RSA* root surface area, *SRL* specific root length

lime-treated plants (Table 3). The roots of non-inoculated lime-treated pea plants were of intermediate length and surface area (Table 3). The SRL allowed us to uncover a distinction between the two substrates based on the presence/absence of the agricultural inoculum. When grown in carbonatite-amended substrates, inoculated plants displayed a significantly higher SRL than non-inoculated plants, whereas a similar comparison between the plants grown in lime did not reveal such a difference (Table 3). Furthermore, the inoculated plants grown in carbonatite-amended substrates had an SRL which was similar to that of plants in both lime treatments (Table 3). The pHs of non-inoculated substrates were comparable (7.07 ± 0.05 and 7.12 ± 0.02 for lime and SRC, respectively). However, when inoculated, the lime-treated substrate had a pH significantly higher (7.35 ± 0.02) than the SRC-treated substrate (7.20 ± 0.04). Plants inoculated with agricultural microorganisms produced nodules, but no significant differences in nodule numbers (average of ~ 22 per root system) or nodule dry weight (~ 7.23 mg total per plant) were seen between amendments. No mycorrhizal fungal colonization of roots was observed. There were no significant differences either in macro- or micro-nutrient contents across treatments (Fig. 3c,d).

Of note is that when treatments were pooled by amendment, not taking into consideration inoculation, plants grown with carbonatite had significantly higher shoot and root biomasses than those grown with lime (Fig. 2b). In addition, carbonatite-treated plants had elevated levels of Ca, Fe, Al, and Mn when compared to lime-treated plants (Fig. 3c, d). However, there were no differences between treatments in terms of R:S ratio (0.48 ± 0.02 for both lime and carbonatite) or root length (1084.68 ± 43.96 cm for lime and 1199.15 ± 37.44 cm for carbonatite). Yet, the SRL of carbonatite-treated plants was significantly lower (81.01 ± 1.81 m g^{-1}) than that of lime-treated plants (86.75 ± 1.60 m g^{-1}). There were no differences in the substrate pH (7.20 ± 0.04 for lime and 7.15 ± 0.02 for carbonatite).

Discussion

The aim of this study was to investigate the effects and mechanisms associated with the use of carbonatite amendment in crop growth. We found that carbonatites can benefit plants in a manner different from that of lime, although the overall responses to carbonatite appear to vary with plant species. In addition, carbonatites appear to impart these benefits depending on various potentially interconnected mechanisms, specifically the interplay between nutrient acquisition, plant root architecture, and soil microorganisms.

Wheat responded to carbonatite more positively than pea in terms of growth and nutrition, especially regarding specific root length, a trait which has been used in the past as an estimate of the overall thickness of roots (Fitter 1985) and, more recently, as an indicator of the P-foraging strategy for herbaceous plants (Hill et al. 2006; Freschet and Roumet 2017; Wen et al. 2019). When either crop was grown with carbonatite, its SRL was altered, and the effect of the carbonatite on this trait was consistently stronger for wheat than for pea. Since wheat and pea have contrasting root architectures (i.e., thin and branched versus thick and coarse, respectively), these data suggest that this trait may be a good predictor of carbonatite

effects on plants. Full root system SRL could thus be used in greenhouse studies with carbonatites and other species or varieties of crops, while in field studies with carbonatites the partial root system SRL could be used (e.g., as with citrus trees, Eissenstat 1991; or with wheat, Corneo et al. 2017).

Here we bring forward strong evidence that carbonatites can improve plant growth, and that their benefits may be largely imparted via a nutrient-based mechanism. It is well established that nutrient availability (or lack thereof) affects root system architecture (e.g., Fitter 1985; Ostonen et al. 2007; Gruber et al. 2013; Freschet and Roumet 2017; Wen et al. 2019). For instance, wheat plants grown in a low-P soil (20 mg kg^{-1}) are known to develop higher SRL to forage in a larger soil volume than plants grown in a high-P soil (200 mg kg^{-1}), which exhibit lower SRL to take advantage of the abundant P resources (Wen et al. 2019). In our study, the lower relative SRL of wheat plants grown with carbonatite matched an increase in shoot P content, suggesting that plants in the carbonatite treatment were capable of acquiring this macro-nutrient through modifications of their root morphology. This would indicate that carbonatite affects wheat growth through some nutrient-based mechanism. However, at this point, it is not possible to separate the potential growth-promoting effects of P from those caused by the other nutrients because of the diversity of nutrients contained in the carbonatite (Sage 1987), and because the shoot contents of several nutrients (e.g., N, P, K, Mn, Fe) were elevated in plants grown with carbonatite. It is also not possible to eliminate the fertilizer used as a source for the nutrients which were of higher content in the shoot. More efficient uptake of P, or other nutrients, from the applied fertilizer may have occurred because of the significant increase in the size of the root systems of the carbonatite-treated plants compared to those of the other treatments. Future studies should be conducted to accurately determine nutrient uptake from the various sources, for example through mass balance calculations in a controlled experimental system, as well as to conclusively identify the cause of the root system growth promotion.

While the SRL of pea has recently been reported as unresponsive to soil P levels (Wen et al. 2019), there is evidence that it increases in the presence of beneficial soil microorganisms which aid in P uptake (Heisinger 1998; Vessey and Heisinger 2001). Here, the SRL of pea was largely similar across all treatments, with the exception of the non-inoculated plants grown with the carbonatite for which the SRL was significantly lower than that of those inoculated plants grown with carbonatite. Although we did not observe differences in shoot nutrient content among the four treatments, it seems likely that the decrease in SRL reflected a change in the interactions between the pea and the carbonatite prompted by the absence of the agricultural soil microorganisms. The connection between pea growth, carbonatites, and soil microorganisms requires further study, especially in light of the apparent effects of carbonatite on soil microorganisms (Jones et al. 2019). It may be that the interactions require more time to establish than was taken here to affect plant growth in a significant manner.

Our data also indicated that the carbonatite exerted an influence on soil CO_2 evolution in wheat. There, the CO_2 evolved from the substrates amended with lime was higher than that of the controls, but no increase was seen with carbonatite. An increase in soil CO_2 evolution with lime application is a common finding attributed to the neutral substrate pH being conducive to microbial growth and to the breakdown of $CaCO_3$ (Fuentes et al. 2006; Holland et al. 2018). In light of this, it is unclear why the carbonatite did not have the same effect given it contains $CaCO_3$ and acts similarly on pH. One explanation, not supported by the data, is that the $CaCO_3$ levels added to the substrate with the lime treatment were different from those added with the carbonatite treatment. Indeed, the carbonatite used here has an approximate liming capacity of 52% (Boreal Agrominerals Inc.) compared to the assumed 100% liming capacity expected of pure calcite. If $CaCO_3$ levels were driving the differences in soil respiration, one would expect the respiration rate of carbonatite-treated soils to be intermediate between lime-treated and control soils, but instead carbonatite and control-amended soils had identical respiration rates. An alternative explanation could lie in the nutrient composition of carbonatite and how it may affect soil microbial composition and function. For example, the carbonatite used here contains approximately 60 ppm of Mn (Jones et al. 2019). For some microorganisms, high levels of Mn are toxic (Gadd and Griffiths 1977), while for others, Mn can be used for metabolic or protective purposes (Ghiorse 1988). Thus, while the increase in pH from

carbonatite addition may be conducive to microbial growth, there may also be unfavourable conditions from the presence of other mineral elements. Finally, the amount of carbonatite exposed to root systems differed between the two trials, and it is possible that the carbonatite minerals were depleted before the CO_2 evolution tests were taken. This may be reflected by the higher Ca content of the carbonatite-treated plant shoots. With the breakdown of calcite, the uptake of Ca by the plant, and the release of CO_2 from the soil, there may not have been enough CO_2 left to be released by organic or inorganic action. However, our finding that carbonatites can produce similar effects as lime but without the accompanying increase in soil microbial respiration certainly warrants further investigation, especially considering the urgent need to reduce global CO_2 emissions. The need for more research in this area is emphasized by recent work on the use of rocks for soil carbon sequestration (Beerling et al. 2018; Haque et al. 2020).

Whether or not the plant-microorganism interactions were important for achieving benefits from the carbonatite appears to be associated with the nutrient acquisition strategy of the plants involved. Crops like wheat, which rely on altering their root morphology to exploit high volumes of soil for nutrient acquisition (Wen et al. 2019), seem to readily acquire nutrients from the carbonatite. So long as soil conditions are conducive to mineral weathering, i.e., pH < 7 and low in calcium (Jones et al. 2020), these crops should obtain significant growth benefits from carbonatite addition. Conversely, crops such as pea that intensely mine a given soil volume and partner with mutualistic soil microorganisms to acquire nutrients (Wen et al. 2019) may require specific conditions, unknown at this time, for carbonatites to be effective rock fertilizers. Another factor requiring examination is the importance of geochemical reactions to the uptake of nutrients, such as the formation of $MnCO_3$ from carbonatite dissolution, or excess soil Ca inhibiting the uptake of B and Zn. Indeed, a recent study by Lambers et al. (2020) suggests that plant-derived carboxylates may help to explain the differences seen between wheat and pea in our system, as they interrelate Mn uptake, P uptake, and root functional types. Such studies would further assist in understanding under which conditions carbonatites benefit crops. Furthermore, the rapid breakdown of the carbonatite, suggested by our study, needs to be demonstrated, and dissolution of carbonatite should be characterized under field conditions. Finally, a confirmation of the significance of grain size towards the release of nutrients from carbonatites is needed.

In conclusion, our results clearly indicate that carbonatites can benefit plant growth and serve as liming agents in agricultural settings. More specifically, we found that the effectiveness of a carbonatite in influencing the growth of a specific crop may be predicted by quantifying the root system SRL, which is directly related to the nutrient-foraging strategy of the crop. For crops that can alter their root morphology to exploit more soil volume (e.g., wheat), carbonatites will be effective at promoting plant growth in situations of low soil fertility. For crops that mine the soil through increased exudation and/or partnerships with soil microorganisms (e.g., pea), the benefits will be more difficult to reap as a growing environment conducive to both breakdown of minerals and growth of beneficial microorganisms will be needed. Future research on carbonatites should focus on how different plant species respond to carbonatite in agroecosystems and whether the presence of certain elements (e.g., Mn) affects the agronomic outcomes. Furthermore, the interactions between carbonatites and soil microorganisms need to be assessed from multiple angles, including mutualisms with plants, mineral weathering and geochemical interactions, and C turnover.

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