# Does a carbonatite deposit influence its surrounding ecosystem?

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## Abstract

Carbonatites are unusual alkaline rocks with diverse compositions. Although previous work has characterized the effects these rocks have on soils and plants, little is known about their impacts on local ecosystems. Using a deposit within the Great Lakes–St. Lawrence forest in northern Ontario, Canada, we investigated the effect of a carbonatite on soil chemistry and on the structure of plant and soil microbial communities. This was done using a vegetation survey conducted above and around the deposit, with corresponding soil samples collected for determining soil nutrient composition and for assessing microbial community structure using 16S/ITS Illumina Mi-Seq sequencing. In some soils above the deposit a soil chemical signature of the carbonatite was found, with the most important effect being an increase in soil pH compared with the non-deposit soils. Both plants and microorganisms responded to the altered soil chemistry: the plant communities present in carbonatite-impacted soils were dominated by ruderal species, and although differences in microbial communities across the surveyed areas were not obvious, the abundances of specific bacteria and fungi were reduced in response to the carbonatite. Overall, the deposit seems to have created microenvironments of relatively basic soil in an otherwise acidic forest soil. This study demonstrates for the first time how carbonatites can alter ecosystems in situ.

Key words: carbonatites, ecosystems, microorganism communities, plant communities, soil chemistry, Spanish River Carbonatite

## Introduction

Carbonatites are a diverse and unusual group of igneous rocks defined by Woolley and Kempe (1989) as containing ≥50% carbonate minerals by volume. There are at least 527 identified carbonatite deposits worldwide, and information on the mineralogy of 477 of these sites is available (Woolley and Kjarsgaard 2008a). The majority (93%) of described deposits have been dated to 2.5–4.0 billion years old (Jones et al. 2013), and these are found in Africa (36%), Asia (34%), and North America/Greenland (23%). Economically, carbonatites are usually considered exclusively as a source of rare earth elements (REE) and trace elements, though several deposits of economic value also contain agriculturally relevant minerals such as calcite ($CaCO_3$) or dolomite ($CaMg(CO_3)_2$) (Woolley and Kjarsgaard 2008b; Myrvang et al. 2016). Carbonatite rocks frequently contain various amounts of the Ca-phosphate mineral apatite and the K-bearing phyllosilicates biotite and phlogopite and

so carbonatites have been considered as an alternative source of these nutrients for plants (Heinrich 1980). Additionally, carbonatites are a source of high-grade phosphate minerals, especially in Brazil and South Africa, and are thus important sources of this element for agriculture (Mariano 1989). More recently, carbonatite rock mined from a deposit in northern Ontario, Canada, has been the focus of preliminary studies exploring its effectiveness as a natural mineral fertilizer (Jones 2016). However, further work is needed to understand what the costs and benefits might be of using minimally processed carbonatite rocks and minerals in agricultural settings.

The impact of these rocks on soil geochemistry has been the focus of several previous studies. Because carbonatite deposits are rich in REE and phosphorus, prospectors have used geochemical fingerprinting in soils and stream sediments to locate buried carbonatite deposits (e.g., Kunzendorf and Secher 1987; Ahn et al. 2014). Vestin et al. (2006) explored the Alnö carbonatite deposit in Sweden with the aim of determining the influence of the alkaline deposit on the chemistry of the overlying soils (0–30 cm depths) and found that there was a distinct geochemical signature characterizing the deposit's influence throughout the surveyed soil horizons. This was followed up by a later study (Vestin et al. 2013) where it was concluded that the Norway spruce (*Picea abies* (L.) H.Karst.) growing in the alkaline soils above the deposit had a higher growth rate and increased tissue Ca content than those trees in less alkaline soils not affected by the deposit. A similar study was done by Myrvang et al. (2016) who investigated nutrient content of the vegetation overlying the Stjernøy carbonatite deposit in Norway. Although the plant content of several elements (Ca, Mg, P, and K) indicated that the vegetation above the deposit was obtaining adequate if not higher levels of nutrients, the ecological significance of this finding was not explored. Hanslinger et al. (2007) reported that that the Alnö carbonatite deposit in Sweden is better delineated by the unique vegetation assemblages supported by the alkaline soils above the deposit than by the existing geological map. This relates to the primary aim of geobotanical prospecting, whereby underlying geological formations are identified based on the overlying vegetation (Brooks 1972). Though difficulties in this technique arise from the expertise required (e.g., to identify the plants) and the sometimes broad habitat preferences of potential indicator species (Moon 2006), it can be used with some success in situations where stark geological boundaries exist. For instance, differences in the types and growth of vegetation above silicate bedrock and carbonatite bedrock were identified in a Finnish study (Talvitie 1979). Although these diverse studies provide evidence that plant growth is improved or altered with carbonatite presence, it is largely unclear how vegetation communities and soil microorganisms may respond to the presence of these rocks. Weathering of minerals from carbonatite deposits increases nutrient content and pH of the overlying soil and it is expected that because of these abiotic changes carbonatite deposits will also influence the biota within the soil and ultimately the larger ecosystems based around those soils. Indeed, in a recent study by Colin et al. (2017), it was demonstrated that when calcite and apatite were introduced to tree stands there were compositional shifts in the soil bulk bacterial community. These minerals are abundant in many carbonatites (Woolley and Kjarsgaard 2008a). Yet, to our knowledge, no study has investigated how the presence of carbonatite rocks affects the overlying soil microbiota.

Here, we examined the Spanish River Carbonatite (SRC) deposit near Sudbury, Ontario, Canada, in its capacity to affect the surrounding ecosystem. More specifically, we investigated whether the SRC causes distinct changes in the biotic and abiotic factors of the ecosystem overlying the deposit by establishing comparisons within and outside the deposit. First, we identified whether the glacial till soils above the deposit were distinct from those present in the area outside the deposit (i.e., whether the soils overlying the deposit had a chemical signature indicative of SRC influence). Second, we assessed whether plant community composition could be explained by differences in soil chemistry in and around the deposit. Finally, we determined the composition of the soil microbial communities, and whether changes based on SRC presence/absence were obvious.

## Materials and methods

### Geological history and deposit description

The SRC deposit is located south of the Spanish River Provincial Park (UTM: 17T 0444556 5163894). The area is part of the Great Lakes–St. Lawrence forest region and contains a mixed forest primarily composed of jack pine (*Pinus banksiana* Lamb), red pine (*Pinus resinosa* Aiton), and trembling aspen (*Populus tremuloides* Michx.). This carbonatite deposit was formed by intrusive igneous activity into quartz monzonite bedrock 1.83 billion years ago (Sage 1987), and the most recent glaciation period in the area was ~13 000 years ago (Dyke et al. 2002). The glacial activity left till of varying depths across the area that somewhat isolates the SRC deposit from the surface. The overburden depth ranges from 0 m at the quarry to at least ~56 m near the outer core according to borehole exploration by Agricultural Mineral Prospectors Inc. (2004). The SRC deposit has an approximate surface area of 3.25 km$^2$, with distinct mineralogical zones (**Fig. 1**; Sage 1987) referred to here as the inner core (IC), outer core (OC), and transition zone (TZ). Based on a geological survey done by Agricultural Mineral Prospectors Inc. (2004), the IC carbonatite is composed primarily of calcitic carbonatite with apatite, abundant to minor amounts of vermiculite, and minor amounts of iron oxide and magnetite. The OC carbonatite consists of calcitic carbonatite interbanded with fenite with abundant to moderate amounts of vermiculite and some iron oxide and apatite. The TZ is predominantly fenite consisting of altered granodiorite with minor amounts of iron oxide and calcitic carbonatite. For this study, two additional zones were designated and used as positive and negative controls, respectively: the quarry (Q) zone within the OC where the SRC deposit is exposed, and the area outside the deposit (OU). Each zone was sampled twice in different areas for a total of 10 sites (**Fig. 1**).

### Vegetation and soil sampling

A modified-Whittaker plot (Stohlgren et al. 1995) was used to assess the species abundance and percent cover of vegetation at each site, including trees. Specific logging/reforestation data were unavailable for the area, and so disturbance was estimated for each site. With the exception of one site dominated by jack pine in the IC that was likely reforested some time ago, the areas surveyed for this project appeared to be either undisturbed or old growth forest. The disturbed site was compared with the other IC site to determine whether the reforestation had altered the vegetation in the area prior to including it in the data set. Sampling was undertaken in mid-August of 2015. Species were identified in the field according to descriptions by Dickinson et al. (2004) and Newcomb (1989). Unknown species were collected and later identified using the Northern Ontario Plant Database (northernontarioflora.ca/), with pressed samples were deposited at the Algoma University herbarium.

Whole root systems were collected from individuals of the three most abundant herbaceous plant species in each plot for assessment of colonization by arbuscular–mycorrhizal fungi. Roots of plants known to be mycorrhizal (following the list by Wang and Qiu 2006) were stained using Trypan blue (Brundrett et al. 1996), and colonization quantified using the gridline intersect method of McGonigle et al. (1990). Soil samples were collected from the centre of each of the modified-Whittaker 10 m$^2$ subplots (two subplots per site). An open-faced auger was used to collect soil until reaching the C horizon or a maximum depth of 80 cm. Aside from the exposed material at the quarry, bedrock material was not encountered. Soil cores were placed sequentially on a sterilized whiteboard and each soil horizon was identified by differences in colour and texture. Where soil horizons could not be visually identified in situ, they were classified according to their placement in the soil profile (e.g., the first non-organic horizon was considered to be horizon A). At the quarry, both exposed bedrock and a residual soil (at least 40 cm deep) were present. The soil had formed directly on the weathered bedrock and consisted of small rock fragments generally <3 mm in size that responded strongly to HCl. This soil was supporting sporadic small plants at the time of sampling, and desiccation cracks were present in low-lying sections. In the soil profile of the sampled areas in the quarry, the

**Fig. 1.** Overview of the Spanish River Carbonatite (SRC) deposit located near the Spanish River in Ontario, Canada. Five zones were considered for the study and are delineated by the black lines: the quarry (grey circle) where SRC is actively mined (Q), the inner core (IC), the outer core (OC), the transition zone (TZ), and the outside of the deposit (OU). Sampling sites are indicated by the white dots. Zones estimated from data provided by Agricultural Mineral Prospectors Inc. (2004). Map data provided by Google (2018) and satellite data provided by DigitalGlobe and CNES/Airbus (2018).

uppermost horizon consisted of SRC (distinguished as weathered by lighter visual appearance) and small amounts ($\leq$0.6%) of organic matter, whereas the lower horizon consisted of unweathered (or less-weathered/darker-coloured) SRC and trace (0.2%) organic matter. For comparisons in modelling, these were labelled as the A and B horizons, respectively. At each site and when possible, two samples were collected from each horizon in sterile 50 mL Falcon™ tubes: one was taken using axenic techniques and stored at 4 °C for analysis of soil microbial community composition (determined by Metagenom Bio Inc., Toronto, Ontario, Canada), and the other was collected for soil chemical analyses (conducted by Actlabs Agriculture Division, Ancaster, Ontario, Canada). The soil chemical parameters determined were pH, organic matter (OM) content, nutrient content (P, K, Ca, Mg, Na, S, Zn, Mn, B, Cu, Fe, Al, and total N), and cation exchange capacity (CEC). The concentrations of these elements were determined by inductively coupled plasma optical emission spectroscopy

following Mehlich-3 extraction. The CEC was calculated from the elemental concentration values, and the total N/OM determined by combustion analysis. Finally, the texture, percent coarse fragments, fizzing response to 10% HCl, and colour of the soils were noted in situ. A total of 78 soil samples were collected for microbial community composition and a total of 75 for soil chemical analyses.

## Microbial community DNA isolation and 16S/ITS sequencing

DNA was isolated from each soil sample using a Norgen Soil DNA Isolation Kit following the supplier's recommendation. For bacteria, the V3–V4 region of the 16S rRNA was selectively amplified using the 340F/806R primers (Takahashi et al. 2014), whereas for fungi the Internal Transcribed Spacer 1 (ITS1) region was amplified using the BITS1/B58S3 primers (Bokulich and Mills 2013). The PCR cycle used for amplification was as follows, with each sample run in triplicate and pooled following amplification: initial denaturing at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 45 °C for 30 s, 68 °C for 50 s and final extension at 68 °C for 10 min. Amplicon size and amount were confirmed by visualizing 2 μL on a 2% TAE agarose gel. Once the amount of 16S/ITS1 rRNA was confirmed, pooled rRNA was gel purified and quantified using Qubit dsDNA HS Assay Kit following the manufacturer's instructions. Library DNA was prepared according to the MiSeq System Denature and Dilute Libraries Guide (Illumina Document #15039740 v01). A 6 pM library with 5% PhiX was sequenced with MiSeq Reagent Kit (v2), and the MiSeq data were streamed to BaseSpace. Although 78 samples were processed, only 71 samples for 16S and 70 samples for ITS were of sufficient quality for successful sequence generation. The mean depth was 25 689 reads for 16S samples and 28 808 reads for ITS samples. Sequences (with primers removed) were assembled with pandaseq (version 2.11; Masella et al. 2012) with a quality threshold of 0.7. Chimera detection/removal and clustering of sequences at 97% identity were accomplished with UPARSE (version 9; Edgar 2013) with singleton sequences removed. The 16S representative sequences were classified using the RDP Classifier (Wang et al. 2007) trained against the Greengenes database (version 13_8) with a 0.8 classification threshold (RDP Classifier bootstrap), whereas the ITS representative sequences were classified against the UNITEdb (version 7.1) using UCLUST (Edgar 2010). The operational taxonomic unit (OTU) representative sequence set was aligned using either the PyNAST algorithm (Caporaso et al. 2010) against the Greengenes (16S) or MUSCLE (version 3.8.31; Edgar 2004; (ITS)) and a representative phylogeny was constructed using FastTree 2.1.3 SSE3 (Price et al. 2010) with the GTR model. Sequence data were deposited in the NCBI database (submission number SUB3462412).

## Statistical analysis and bioinformatics

Our first question was whether the chemistry of soils within the deposit was distinct from that of the soils outside of the deposit, and possessed a chemical signature indicative of SRC influence. Because a large number of soil chemical variables had near-linear or linear distributions, a principal component analysis (PCA) was used to identify differences in chemistries across the sampled areas. For the PCA, the soil horizons were not considered (individual horizon data is available in Table S1). This test was followed by analyses of variance (ANOVAs) to validate whether soil chemical factors could be used to group samples according to presumed SRC influence. Post hoc Tukey's HSD tests were conducted to confirm differences in soil chemical properties across groups. Our next question was whether differences in plant community composition could be explained by observed differences in soil chemistry. To answer this question, constrained ordination with redundancy analysis (RDA) was undertaken with the presence/absence data of plant species from the 1 m² modified-Whittaker subplots (excluding mature trees). Data from the Q sites were not included in this analysis because the area is actively mined and therefore highly disturbed. Because soil chemical analyses were not conducted for the 1 m² subplots, the mean values from the 10 m² subplots were used. Analysis was done using the R "vegan" package (version 2.4-1; Oksanen et al. 2016), specifically the "rda" function for RDA and the "anova.cca" function (1500 permutations) for the random permutation test. Additionally,

the Shannon diversity indices were calculated (Fowler et al. 1998) for each sub-plot in the modified-Whittaker plot.
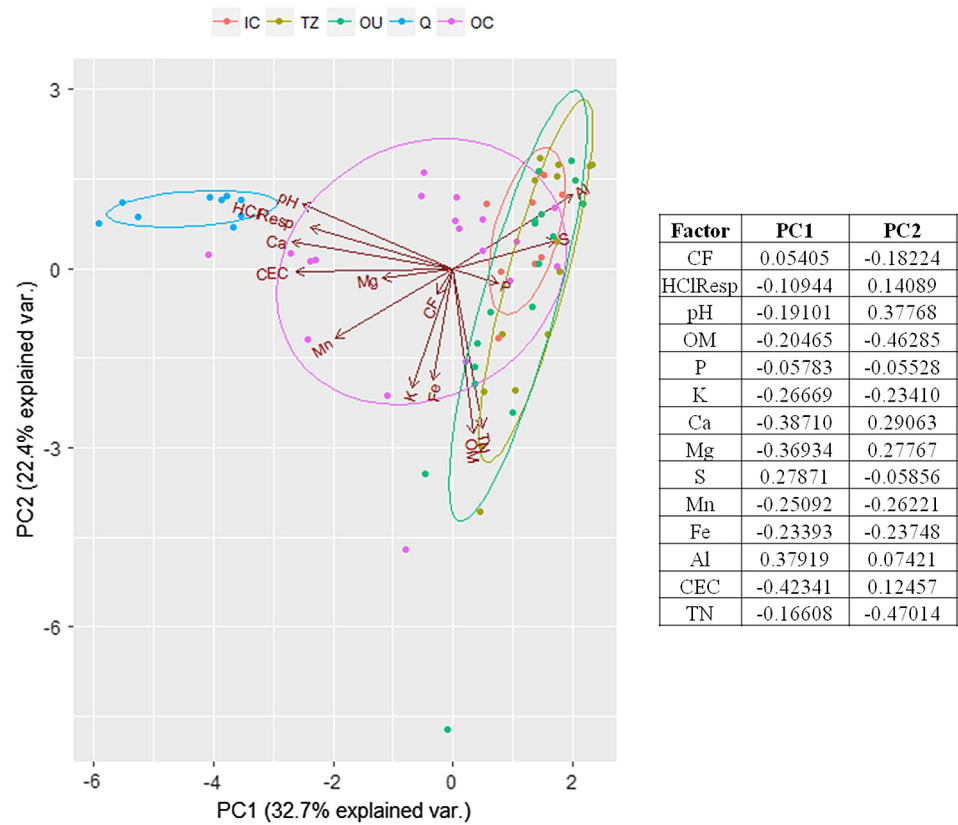
Our last question was whether differences in soil chemistry affected bacterial (16S) and fungal (ITS) communities, which was addressed using two methods: non-metric multi-dimensional scaling (NMDS) coupled with a permutational multivariate analysis of variance (PERMANOVA) to quantify changes in community composition, and differential abundance analysis to identify indicator OTUs. Bacterial and fungal community composition was determined with core samples pooled (i.e., without considering horizons). Prior to the analysis, OTUs with low read numbers (<5) were removed and abundances converted to relative proportions. Differential abundance was evaluated using the DeSeq2 R package (version 1.12.4; Love et al. 2014), with the geometric means correction for data containing zeros. The PERMANOVA analysis was undertaken using the vegan R package) and the "adonis" function with a Bray–Curtis distance matrix. When possible, the differentially abundant OTUs were further classified by an NCBI nucleotide BLAST search for highly similar sequences against the NCBI nucleotide collection (≥99% similarity) excluding uncultured and environmental sequences. All data analyses were conducted in the R software environment (version 3.3.1; R Core Team 2016).

## Results

### Soil chemistry differed with overburden depth and only weakly across zones

It was expected that the different mineralogy of bedrock in the zones within the SRC deposit would give rise to unique soil chemical parameters in each of these areas. Although the sampling sites partially grouped by zone in the PCA (Fig. 2), some areas within the deposit (e.g., IC) had nearly indistinguishable soil chemistries from those areas outside the deposit. Overall, soils from the IC, TZ, and OU zones grouped together, the Q soils were the most different, and the OC soils grouped between those two sets.

According to the ordination vectors and the loadings for the main axis of sample separation (Fig. 2; PC1), the primary differences among soil samples, in order of contribution to PC1 and irrespective of sign, were seen in CEC, Ca, Al, Mg, S, Mn, pH, and P. Using the differences in these parameters, a SRC influence signature was established and used to reclassify the sampling areas into three new groupings: areas of high SRC influence (Q), areas of moderate SRC influence (OC), and areas of low/negligible SRC influence (IC, TZ, OU). These groups were validated by comparing the soil found outside of the deposit (low/negligible influence) to the "pure" SRC at the quarry (high influence) using a one-way ANOVA as above (Fig. 3). Areas of high SRC influence were characterized by increased levels of Ca, Mn, and higher CEC and pH (Figs. 3a–3d, respectively) when compared to low/negligible influence sites. The moderate influence sites were most similar to the high influence sites with regards to those elements, but usually did not reach the same levels. When assessed across horizons, soil pH was typically within 0.2 units of any other horizon within influence categories (Table 1). There was, however, a stark difference in soil pH between the high, moderate, and low/negligible SRC sites, with them being basic (~pH 7.60), moderately acidic (~pH 5.85), and acidic (~pH 4.85), respectively (Table 1). The areas of low SRC influence were characterized by increased Al and S content compared to those placed in the moderate and high categories (Figs. 3e and 3f, respectively). Finally, the soil Mg (Fig. 3g) and P (Fig. 3h) contents were not indicative of influence category. The soils across sites could not be differentiated based on their physical characteristics; they had a similar texture, HCl response, and colour, with the exception of the soil at the Q sites. Following the above results, the SRC influence categories were used for the later analyses where applicable (e.g., PERMANOVA). The soil chemistry of the IC site that was thought to have been disturbed by reforestation was not different from that of the other IC site sampled.

| Factor | PC1 | PC2 |
|--------|---------|---------|
| CF | 0.05405 | -0.18224 |
| HClResp | -0.10944 | 0.14089 |
| pH | -0.19101 | 0.37768 |
| OM | -0.20465 | -0.46285 |
| P | -0.05783 | -0.05528 |
| K | -0.26669 | -0.23410 |
| Ca | -0.38710 | 0.29063 |
| Mg | -0.36934 | 0.27767 |
| S | 0.27871 | -0.05856 |
| Mn | -0.25092 | -0.26221 |
| Fe | -0.23393 | -0.23748 |
| Al | 0.37919 | 0.07421 |
| CEC | -0.42341 | 0.12457 |
| TN | -0.16608 | -0.47014 |

**Fig. 2.** Principal component analysis of soil chemistry factors coloured by zone. The table to the right contains the loadings of each soil factor to the first two components. IC, inner core; TZ, transition zone; OU, outside of the deposit; Q, quarry; OC, outer core; CF, coarse fragments (%); OM, organic matter content (%); CEC, cation-exchange capacity (meq/100 g); TN, total nitrogen (%).

## Plant community composition varied with soil chemical properties

Separation of plant communities in the RDA was driven by increases in pH, Ca, Mg, CEC, and Mn (**Fig. 4a**). Constraining the ordination by soil chemical factors explained 26.0% of the variation in community composition, and all the tested soil factors were shown to equally contribute to community variation as shown by the permutation test output (**Fig. 4b**). In sites of moderate SRC influence (i.e., within the OC), the abundant plant species (i.e., defined as ≥3 individuals across the two tested sites within a zone) consisted of *Acer spicatum* Lamarck, *Solidago altissima* Linnaeus, *Thuja occidentalis* Linnaeus, *Acer rubrum* Linnaeus, *Rubus idaeus* ssp. *strigosus* (Michaux) Focke, and *Comptonia peregrina* (Linnaeus) Coulter. In sites of low/negligible SRC influence (i.e., the IC, TZ, and OU), the plant species that were abundant (i.e., found in at least two of the zones) and unique to these areas consisted of *Maianthemum canadense* Desfontaines, *Vaccinium angustifolium* Bentham, *Pleurozium schreberi* (Bridel-Brideri) Mitten, *Pteridium aquilinum* (Linnaeus) Kuhn, and *Lysimachia borealis* (Rafinesque-Schmaltz) Manns & Anderberg. Although several abundant species were present in both the moderate and low/negligible SRC influence sites, the only abundant species present across all areas was *Cornus canadensis* Linnaeus, which had approximately 10 individuals in each of the IC, TZ, and OU, but only three individuals in the OC sites. The Shannon-diversity indices for the 1 $m^2$ subplots suggested an increase in plant diversity/abundance as SRC influence decreased (**Fig. 4c**). As the most abundant species in each zone were typically different,
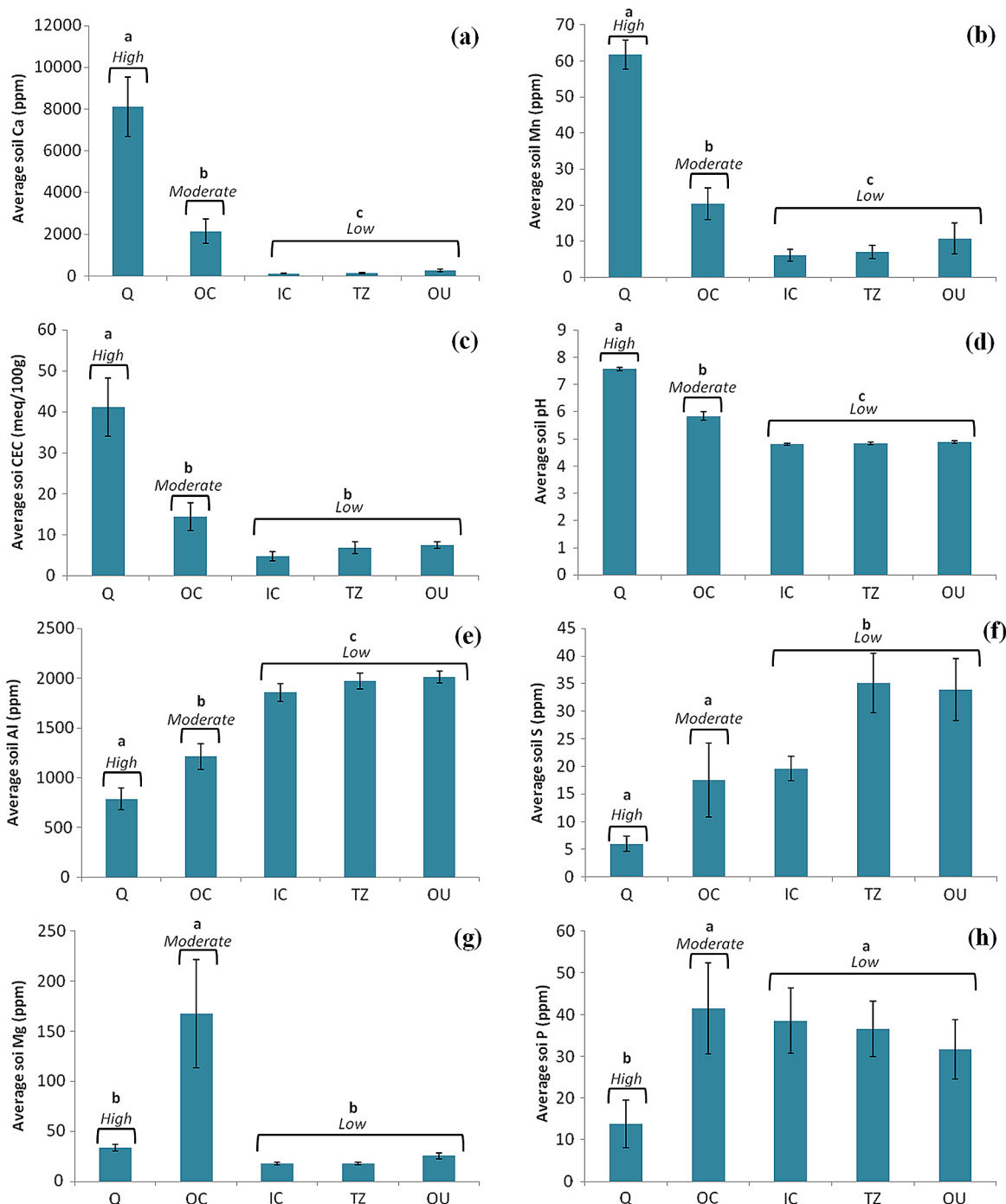
**Fig. 3.** Mean soil chemistry values across zones and high, moderate, and low/negligible Spanish River Carbonatite (SRC)-influence categories. Deposit zones were assigned to influence categories based on similarities in their soil chemistry values (confirmed by statistical analysis) and are indicated by the above brackets. The Ca, Mn, S, Mg, and P values were log-transformed before statistical analysis. Chemistry values that differ significantly between SRC-influence categories (one-way ANOVA, 95% confidence level) are indicated with different lower case letters. CEC, cation-exchange capacity.

**Table 1.** Mean (± standard error) soil pH for each of the major soil horizons (A, B, C) separated by low/negligible, moderate, and high Spanish River Carbonatite (SRC)-influence.

| Soil horizon | Low | Moderate | High |
|---|---|---|---|
| A | 4.80 ± 0.06 | 5.80 ± 0.27 | 7.60 ± 0.07 |
| B | 4.85 ± 0.04 | 5.85 ± 0.12 | 7.63 ± 0.12 |
| C | 4.89 ± 0.04 | 5.88 ± 0.33 | 7.40 ± 0.10 |

**Note:** $n$ = 2–11 samples per SRC-influence category per horizon.
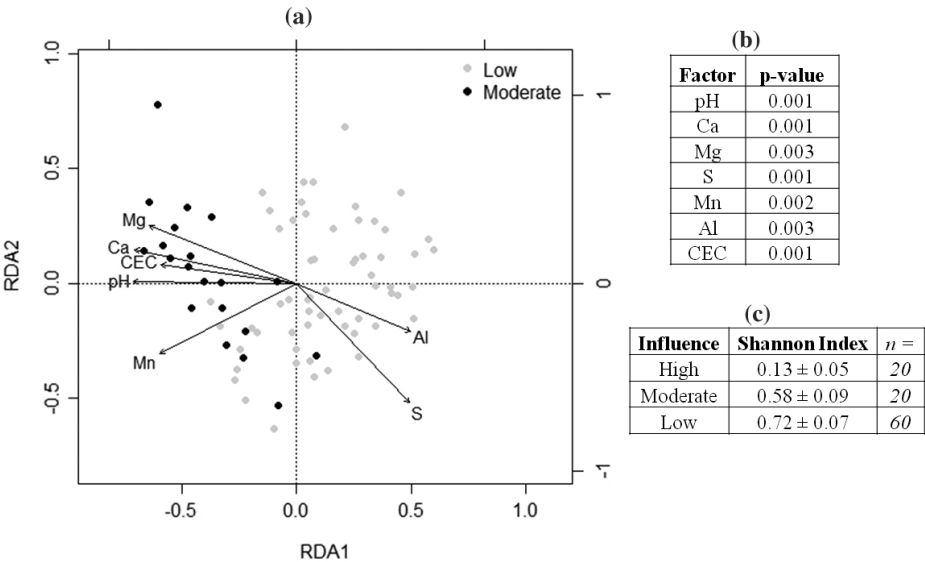


**Fig. 4.** (a) RDA biplot with samples shaded by Spanish River Carbonatite (SRC)-influence categories. Species presence/absence from the 1 m$^2$ modified-Whittaker subplots were used as the vegetation data, and the averaged soil chemistry variables from the 10 m$^2$ subplots used as the environmental chemistry data. The percent variation explained by the soil factor constraints is 26.0%. (b) Results of the soil chemistry permutation test to determine significant contributions to plant community structure. For the permutation test, 1500 permutations were used with "set.seed(2)". (c) Average Shannon diversity indices from the 1 m$^2$ subplots with the standard error and number of samples for each SRC influence category. CEC, cation-exchange capacity (meq/100 g).

meaningful comparisons of mycorrhizal colonization could not be conducted (Table S2). Analysis of the growth of mature trees also could not be completed because the dominant tree species in each area were typically different (Table S3); it was further complicated by possible reforestation. The vegetation community of the disturbed site did not appear different from that of the other IC site during ordination.

## SRC impacted specific microorganisms but not overall communities

When all horizons were pooled, the bacterial communities showed weak grouping by SRC influence category (Fig. 5a). The PERMANOVA indicated a significant influence of SRC ($p$-value = 0.021) on bacterial community composition, although the variation explained by this model was very low ($R^2$ = 0.0766). Between the high and moderate SRC influence sites, no bacterial OTUs were found to be significantly different in abundance, and between the moderate and low categories, only one OTU was significantly more abundant in the sites of moderate influence. This OTU was identified
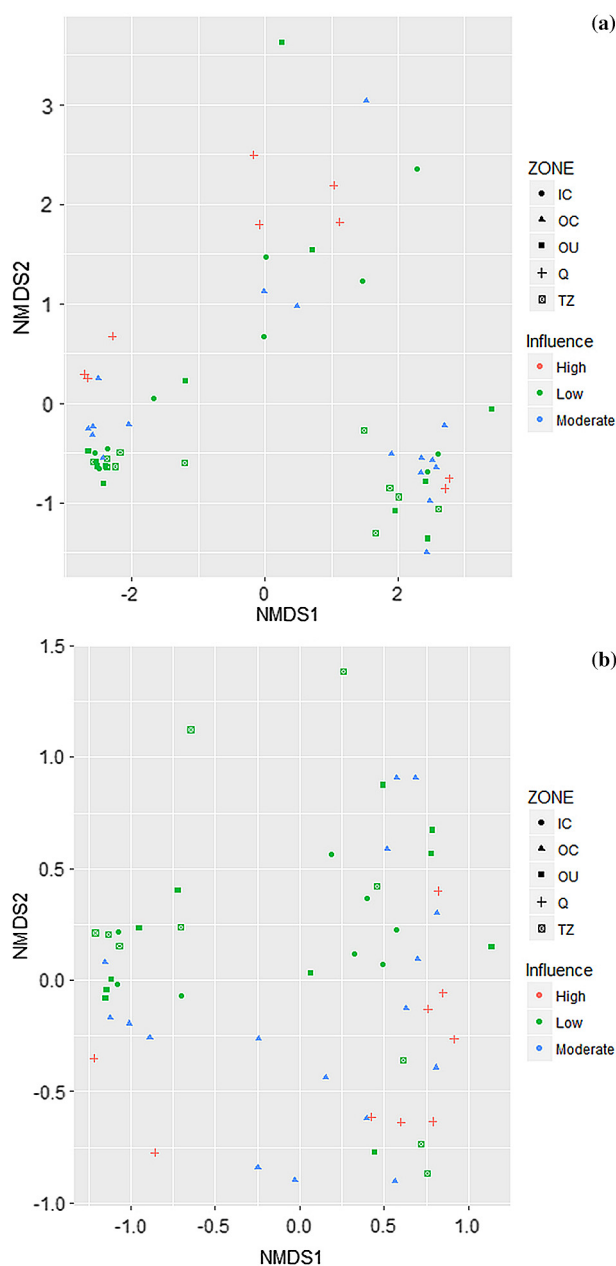
**Fig. 5.** Non-metric multidimensional scaling (NMDS) biplots of 16S bacterial (a) and ITS fungal (b) community data for all horizons pooled together. Samples are colour-coded by Spanish River Carbonatite (SRC) influence level. IC, inner core; OC, outer core; OU, outside of the deposit; Q, quarry; TZ, transition zone.

to the Micrococcaceae family (Phylum Actinobacteria). The BLAST search was able to further narrow down the identity of this OTU, with 100% identity match to several species within both the *Arthrobacter* and *Pseudoarthrobacter* genera ($e$-value = 0.0). Between the low and high SRC influence sites, there were a total of 28 bacterial OTUs that were of differential abundance (**Table 2**). Only two of these OTUs, the aforementioned Micrococcaceae and a Gaiellaceae family member (Phylum Actinobacteria), were more abundant in sites of high SRC. The rest were of higher abundance in the

**Table 2.** Bacterial OTUs of differential abundance between high and low/negligible Spanish River Carbonatite (SRC) sites.

| Phylum | Class | Order | Family | Genus | Abundance with SRC |
|---|---|---|---|---|---|
| Actinobacteria | Thermophilia | Gaiellales | Gaiellaceae | NA | Increased |
| Actinobacteria | Actinobacteria | Actinobacteriales | Micrococcaceae | NA | Increased |
| AD3 | ABS-6 | NA | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | DA052 | Ellin6513 | NA | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Acidobacteriaceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Acidobacteriaceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Acidobacteria | Acidobacteriia | Acidobacteriales | Koribacteraceae | NA | Decreased |
| Actinobacteria | Actinobacteria | Actinomycetales | NA | NA | Decreased |
| Proteobacteria | Alphaproteobacteria | Rhizobiales | Bradyrhizobium | *Bradyrhizobium* sp. | Decreased |
| Proteobacteria | Betaproteobacteria | Burkholderiales | Burkholderiaceae | *Burkholderia bryophila* | Decreased |
| Proteobacteria | Alphaproteobacteria | Rhodospirillales | Rhodospirillaceae | NA | Decreased |
| Proteobacteria | Alphaproteobacteria | Rhodospirillales | Rhodospirillaceae | NA | Decreased |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Sinobacteraceae | NA | Decreased |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Sinobacteraceae | NA | Decreased |
| Proteobacteria | Gammaproteobacteria | Xanthomonadales | Sinobacteraceae | NA | Decreased |
| Proteobacteria | Gammaproteobacteria | Xanthomonodales | Sinobacteriaceae | NA | Decreased |

**Note:** NA, not applicable.

low/negligible SRC influence sites. For the Gaiellaceae, the BLAST search narrowed the identity down to *Gaiella occulta* strain F2-233 (95% identity, *e*-value = 0.0, accession: NR118138.1).

For the fungi, it was unclear whether there was any grouping based on SRC influence level (**Fig. 5b**). Though the PERMANOVA indicated that SRC was a significant driver of community differences ($p = 0.032$), the variation explained by the model was quite low ($R^2 = 0.04983$). Several fungal OTUs were found to be differentially abundant between the SRC influence categories: a *Tylospora* species (Phylum Basidiomycota), a Thelephoraceae species (Phylum Basidiomycota), a Leotiomycetes

member (Phylum Ascomycota), and two OTUs that could not be assigned to a class rank. A BLAST search was able to identify one of the unassigned OTUs to several *Ascomycota* sp. (100% identity, *e*-value 2e-87). For the other OTUs, the BLAST search narrowed the identity of the *Tylospora* sp. to *Tylospora asterophora* (99% identity, *e*-value = 1e91, accession: JQ711985.1), the Thelephoraceae member to a *Tomentella* sp. (97% identity, *e*-value = 2e90, accession: MH248057.1), and the Leotiomycetes member to the Helotiaceae family (100% identity, *e*-value = 2e80 for several *Meliniomyces* and Helotiaceae species). One unassigned OTU could not be identified with any certainty and was disregarded. Generally, fungal OTUs were less abundant in areas of moderate/high SRC influence than in those of low influence. *Tylospora asterophora*, *Tomentella* sp., and the member of the Helotiaceae were more abundant in the low influence sites than in moderate sites; counts of these OTUs and an *Ascomycota* sp. were also higher in the low influence sites than in the high influence sites. Between the moderate and the high influence sites, the *Ascomycota* sp. was more abundant in the former, whereas the *Tomentella* sp. was more abundant in the latter.

## Discussion

This is the first comprehensive study investigating how the soil properties associated with a natural carbonatite deposit influence the plant and soil microbial communities in a Great Lakes–St. Lawrence forest ecosystem. As with most of this part of North America, the deposit is covered by a layer of overburden from past glaciation; however, despite this it was found that some areas above the deposit had an identifiable soil chemical signature indicative of SRC. The variable soil chemistry resulted in differences in plant community structure. This was demonstrated in areas of moderate SRC influence that had different plant species than those areas of low/negligible influence. However, the influence of SRC did not appear to be a dominant factor in shaping soil microbial communities. Nevertheless, several bacterial and fungal OTUs were found to be differentially abundant between areas of high and low/negligible SRC influence.

### SRC, soils, and the influence of the deposit on the overlying ecosystem

Several factors indicated an influence of the SRC deposit on soil chemistry, as revealed by a comparison between zones. Because CEC, Ca, Al, S, Mn, and pH values of the OC soils (moderate influence) were often more similar to those of the exposed SRC comprising the Q samples (high influence) than to those of other zones (low/negligible influence), the increases in these parameters were considered to be a signature of SRC. The primary mineral of SRC is calcite, and like many other carbonatite deposits it is accompanied by a variety of nutrient-bearing accessory minerals such as biotite and apatite (Sage 1987). Despite there being an overburden layer of variable depth (0 m at the quarry to at least 56 m elsewhere) covering the deposit, it appears that these minerals are affecting some of the sampled soils. Kunzendorf and Secher (1987) made a similar suggestion for the Qaqarssuk carbonatite deposit in Greenland, which is covered by soils formed from alluvial deposits that are enriched in Nb and P originating from the underlying carbonatite. Thus, it can be inferred that in some areas the depth of the overburden is shallow enough that weathered SRC exerts an effect on the soils. This needs to be validated as there is not a reliable overburden map for the whole deposit. It is assumed that weathering of SRC fragments and their incorporation into the soils is responsible for the SRC signature identified in the OC sites surveyed here. This assumption is justified based on the similar soil chemistry between OC and Q sites, and by previous works demonstrating carbonatite influence on soil chemistries (e.g., Kunzendorf and Secher 1987; Vestin et al. 2006; Ahn et al. 2014). However, this study has not verified the presence of carbonatite minerals in the soil outside of the quarry (Q). Although the soil chemistry of the OC sites was similar to that of the Q, many values were significantly lower in the OC and it is likely that the overburden present in the OC dilutes the SRC influence. Additionally, topography, hydrology, and vegetation presumably play important roles in the transfer

of carbonatite elements into the glacial till soil. For instance, where the deposit is near the surface at higher elevations, a signature of SRC might be found both in soils at these locations and in nearby topographical lows where downward water movement through the soils would have transported some minerals or elements. Nutrient translocation by the action of plants could also have occurred and a mechanism for this is provided by the nutrient-uplifting hypothesis of Jobbágy and Jackson (2004), which posits that trees (and other vegetation) are active in transporting lithosphere-derived nutrients to the surface of soils. This translocation occurs when lithosphere nutrients are incorporated into above- and below-ground plant biomass, and subsequently released when the plant organic matter decomposes close to the soil surface.

The soil chemistry differences above the deposit indicate that in some areas SRC has had a strong influence on the soils. Above-ground differences in plant community composition are therefore likely linked to changes in soil chemistry. Several plant species unique to the moderate influence sites (i.e., the OC) are typically those colonizing areas post-disturbance or that are tolerant to alkaline soil pH. For instance, *C. peregrina* has been considered a weed, and post-fire disturbance conditions are favourable for its growth (Hall et al. 1976). As none of the outer core sites displayed signs of recent disturbance (e.g., being dominated by younger trees or displaying signs of fire damage on older wood), it is likely that the abundance of ruderal species in the OC is not due to a disturbance event but to the presence of SRC. With the stark differences in soil pH between areas of moderate and of low/negligible SRC influence, it appears that one of the main effects of the deposit has been the creation of ecological microhabitats ("islands") of more basic soil pH in what would otherwise be a large surrounding area ("sea") of more acidic soils. The more basic the soils, the greater likelihood they will be colonized by species tolerant of soil pH changes, i.e., ruderal species. Based on the Shannon diversity indices, where areas of moderate influence were seen to have lower indices than those areas without SRC influence, we hypothesize that the altered soil chemistry caused by SRC constrains the abundance and diversity of the plant species present in these areas. The data collected regarding tree species show the same trend whereby in moderate influence sites there were fewer tree species and fewer individuals than in low/negligible sites (**Table S3**). This might be due to the soil chemistry restricting the types and number of plant species and (or) the spatial separation from areas where more alkaline-adapted species are present and could serve as seed sources. These species could also be less tolerant of the higher, potentially toxic Al concentrations found in the more acidic soil conditions. A similar situation whereby the bedrock and soil chemistry controlled the distribution of plant species was found in a broad survey of rock habitat vegetation performed by Tyler (1996). To verify this hypothesis, the soil pH across the deposit area could be assessed, and the areas of higher pH (and thus presumably under carbonatite influence) identified and surveyed for vegetation community composition. Such a study would also assist in determining the magnitude of overburden depth and hydrology/topography in expressing the effects of the carbonatite on the deposit area soils.

Although it was expected that the soil microbial communities would be significantly shaped by differences in pH or by the abundance of elements provided by the weathering of SRC's nutrient-bearing minerals, the data did not support this hypothesis. For instance, many species of bacteria that have been associated with the weathering of carbonatite minerals (e.g., *Burkholderia* (Uroz et al. 2011) or *Pseudomonas* (Colin et al. 2017)) were not more abundant in the moderate- or high-influence sites than in the low/negligible sites. The only bacterial OTU consistently in higher abundance in SRC-affected areas was tentatively identified as a member of the genus *Arthrobacter* belonging to the family Micrococcaceae. Members of this genus have been shown to utilize a diverse set of carbon/nitrogen sources (Hagedorn and Holt 1975). Furthermore, their ability to metabolize aliphatic amino acids and aromatic hydrocarbons (Hagedorn and Holt 1975), as well as their nitrification activity, were found to be higher at more neutral than acidic pHs (Brierly and Wood 2001). The ability to utilize a variety of nutritional substrates and to nitrify under the more basic soil conditions in SRC-affected

areas would be beneficial to this microbial group as SRC does not contain appreciable amounts of N, and these characteristics could explain the higher presence of *Arthrobacter* in areas with the SRC signature. This would be especially important in the Q, where the soils almost exclusively consist of SRC particles. In terms of the fungal OTUs, most were found to decrease in abundance with increasing SRC influence. This is in agreement with the observations made by Rousk et al. (2010) that fungi prefer acidic soils whereas bacteria prefer neutral/alkaline soils.

The most abundant bacterial families detected in our soil samples were either saprotrophic and (or) associated with the rhizosphere of plants (Table S4). Similarly, aside from the Pseudeurotiaceae, most fungal families were saprotrophic and (or) ectomycorrhizal (Table S4). The identity of tree species dominant in a given area can be a strong determinant of fungal community composition (Urbanová et al. 2015). As such, differences in leaf litter composition and (or) mycorrhizal hosts may provide an explanation for the differences seen in fungal abundances between low/negligible and the high or moderate SRC influence soils. Additionally, increasing the pH to values similar to those observed in the moderately-influenced sites has previously been reported to negatively affect the survival of ectomycorrhizae on *P. abies* roots (Lehto 1994). This suggests that the increased soil pH resulting from the presence of SRC may not be conducive to ectomycorrhizal colonization of the tree species at the deposit. Given our focus in this study on the assessment of the colonization of herbaceous plants by arbuscular mycorrhizal fungi, the colonization of trees by ectomycorrhizal fungi needs to be investigated in future studies.

## Conclusion

The results reported here are the first that show that a carbonatite deposit can contribute to shaping the overlying ecosystem. We detected a SRC signature in some soils, which had an effect on both the plant and soil microbial communities. Furthermore, using Illumina Mi-Seq, we were able to investigate both community-level and individual OTU-level responses of microbes to SRC in the deposit. Overall, it appears that when the overburden is shallow enough to allow for the carbonatite deposit to influence the soil chemistry, pockets of more basic soils have developed in an area of otherwise predominantly acidic soils. In turn, this has constrained the abundance and diversity of plant species in these areas and affected the abundance of several microorganisms. However, most groups of bacteria and fungi in communities were unaffected by the SRC. Based on these findings, in areas where carbonatite deposits are found close to the surface, it can be expected that ecological effects may emerge, which may or may not lead to unique ecosystem-level features. As such we recommend that ecosystems in and around these deposits should be studied using similar approaches to those reported here to better understand these sites and, if needed, develop appropriate strategies for environmental protection should these areas be targeted for mining. Finally, although the soil properties, plants, and soil microorganisms were emphasized here, impacts on other relevant organisms (e.g., insects or other fauna) should be included in future work.

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## Author contributions

JMCJ, PMA, and FCG conceived and designed the study. PMA and FCG contributed equally to the work. JMCJ, MDJL, and TCC performed the experiments/collected the data. JMCJ, EAW, MDJL, PMA, and FCG analyzed and interpreted the data. TCC, PMA, and FCG contributed resources. JMCJ, EAW, PMA, and FCG drafted or revised the manuscript.

**FACETS**

## Competing interests

The authors have declared that no competing interests exist.

## Data availability statement

All relevant data are within the paper and in the Supplementary Material. Unknown species were collected and later identified using the Northern Ontario Plant Database (northernontarioflora.ca/), with pressed samples deposited at the Algoma University herbarium.

## Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2018-0029.

Supplementary Material 1

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