SHORT COMMUNICATION

Carbon sequestration in artificial silicate soils facilitated by arbuscular mycorrhizal fungi and glomalin-related soil protein

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Abstract
In urban areas, pre-existing concrete-based demolition wastes and purposely introduced crushed dolerite have been used to create artificial soils, which capture carbon (C) as carbonate minerals and offset greenhouse gas (GHG) emissions. Arbuscular mycorrhizal fungi (AMF) can enhance capture of C in artificial soils through production of glomalin-related soil protein (GRSP), which facilitates soil organic carbon (SOC) storage and aggregation, and may also enhance precipitation of soil inorganic carbon (SIC). In this paper, we show how different mixtures of dolerite and concrete affect AMF colonization and community structure, contents of easily-extractable and difficultly-extractable GRSP, and soil organic and inorganic carbon contents. We used nine demonstration plots, 1 m deep, to simulate a constructed urban soil, consisting of different proportions (0, 30, 50, 70 and 100%) of either crushed concrete demolition waste or dolerite quarry fines and sown to a species-rich meadow mixture, to investigate AMF colonization and community structure (using DNA terminal restriction fragment length polymorphism), contents of easily-extractable and difficulty-extractable GRSP, and both organic and inorganic carbon contents. All artificial soils supported functioning AMF communities with different levels of GRSP, SIC and SOC. The 100% dolerite and 100% concrete soils had higher values of difficulty-to-extract GRSP and SIC than pure sand, whereas 100% concrete had higher AMF colonization and SOC than sand. AMF community analysis indicated that high GRSP producing species were abundant in 100% dolerite and 100% concrete. These findings demonstrate that there is potential to incorporate demolition waste or dolerite products into the land to support environmental sustainability and enhance soil C sequestration.

Highlights
- In constructed soils, crushed concrete and dolerite more effectively enhance GRSP and soil organic and inorganic carbon contents than sand.
- Use of crushed concrete and dolerite in plant-growing substrates is a novel way to combat climate change.
Soil carbon (C) sequestration has gained attention as a mechanism for offsetting carbon dioxide (CO₂) emissions by capturing atmospheric CO₂ and storing it in terrestrial systems in stable forms. Although storage of C in soil organic pools has generated the majority of interest in recent years (e.g., Lal, 2004; Minasny et al., 2017), C can also be sequestered as soil inorganic carbon (SIC). SIC, a highly chemically stable form, is a precipitated carbonate for which the turnover time exceeds 85,000 years, compared to 200–1,200 years for SOC (Bugbio et al., 2015; Cerling, 1984). SIC sequestration occurs when divalent cations such as calcium (Ca²⁺) and magnesium (Mg²⁺) from calcium-bearing silicates such as wollastonite (CaSiO₃) react with dissolved CO₂ to form carbonate minerals (Colbourn, Ridgwell, & Lenton, 2015; Manning, Renforth, Lopez-Capel, Robertson, & Ghazireh, 2013).

Natural basic silicate rocks (e.g., basalt or dolerite) or artificial calcium- (Ca) and magnesium- (Mg) rich materials (e.g., concrete) contain high contents of silicates and show potential for enhancing SIC sequestration (Matichenkov & Bocharnikova, 2001). It is estimated that the world’s current production of Ca-bearing silicate wastes (e.g., construction and demolition waste, furnace slag, coal fuel ash and mine waste) could sequester 0.19–0.33 Gt C year⁻¹ globally, which is equivalent to 24% of the target for reducing atmospheric CO₂ to keep the global temperature increase to below 1.5 °C by 2,100 (Renforth, Washbourne, Taylder, & Manning, 2011). Furthermore, using these materials for C sequestration in soils will not only mitigate CO₂ emissions, but also divert wastes from landfills towards an environmentally positive outcome (Renforth et al., 2011). Use of crushed rock for climate mitigation as well as remineralization is therefore growing globally (Beerling et al., 2020; Manning & Theodoro, 2020).

The biotic and abiotic factors that impact C sequestration in SOC and SIC are not yet well understood. Arbuscular mycorrhizal fungi (AMF) may play an important role in enhancing sequestration via the production of glomalin-related soil protein (GRSP) (Rillig & Mummey, 2006) by AMF hyphae, which stabilizes soil aggregates and protects the sequestered C within them (Wilson, Rice, Rillig, Springer, & Harnett, 2009; Wright & Upadhyaya, 1996). Moreover, GRSP itself represents a stable fraction of SOC that contributes to long-term storage of C in soils.

Many studies have focused on the individual effects of AMF, GRSP and silicate soils on C sequestration, but none have examined how AMF and GRSP vary between different mixtures of artificial silicate soils (e.g., dolerite fines and demolition concrete waste), and how this relates to C sequestration in systems with established plant communities (Kumar, Singh, & Ghosh, 2018; Manning et al., 2013; Rillig, 2004; Washbourne, Renforth, & Manning, 2012; Wright & Upadhyaya, 1996). Indeed, the ability of artificial soils based on these materials to support a diverse microbial community is not known. This study addresses these knowledge gaps by exploring relationships between AMF diversity and community structure in artificial soils and the plants they support, and the content of GRSP, SOC and SIC in these soils.

In this study we used established (May 2015) demonstration plots (Figure 1) of artificial soils planted to a species-rich meadow mixture (http://wildseed.co.uk/mixtures/view/54) at Cockle Park Farm near Morpeth, Northumberland (UK; 55°12’ N, 2°18’ W). There are 14 plots in total, each 3 m wide by 4 m long, originally excavated to 1 m and filled with a substrate representing different mixtures of sand, crushed dolerite and crushed concrete. Soils and roots of red fescue in a subset of nine plots (2, 4, 5, 6, 7, 8, 10, 12, 14) representing 0, 30, 50, 70 and 100% mixes of sand and dolerite or concrete were sampled in triplicate from each plot in June 2018. Both the soil and roots of red fescue (Festuca rubra) were extracted to a depth of 20 cm. Typically, red fescue has a shallow root system, which is confined to the topsoil layer, with about 70% of the root biomass concentrated within the top 20 cm (Brown, Percivalle, Narkiewicz, & DeCuollo, 2010). We considered 0.7 m unmixed dolerite (Plot 5) and 0.7 m unmixed concrete (Plot 7) as either 100% dolerite or 100% concrete, because the sample was from that part of the soil profile composed entirely of crushed concrete or dolerite; in these plots, the sand layer was well below the maximum sampling depth.

AMF colonization was assessed using ink-vinegar staining techniques (Vierheilig, Coughlan, Wyss, & Piche, 1998). Based on the scoring system in McGonigle, Miller, Evans, Fairchild, and Swan (1990), the occurrences of intersections with hyphae, arbuscules or vesicles were deemed positive indicators of colonization and recorded. Terminal restriction fragment length polymorphism (T-RFLP) using AMF-specific primers as described in Gollotte, Tuinen, and Atkinson (2004) was used to characterize the AMF community in the roots and soils, using TaqI as the restriction enzyme (Mummey & Rillig, 2008). This analysis was conducted on duplicate...
samples of soil and roots from each plot randomly selected from the initial set of three samples. The size distributions of fragmented DNA were detected using an Applied Biosystems 3,730 DNA Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) with ROX-500 as the size standard. Genemapper software (Applied Biosystems) was used to identify peak sizes and areas under each peak (terminal restriction fragment (T-RF)). Easily extractable GRSP (EE-GRSP) and difficultly-extractable GRSP (DE-GRSP) were quantified based on the protocols in Wu, Cao, Zou, and He (2014). A modified Lowry assay (Ohnishi & Barr, 1978) was used to quantify the proteins (Total Protein Kit, Micro Lowry, Onishi & Barr Modification; Sigma-Aldrich, St. Louis, Missouri, USA). SIC was measured using a Scheibler calcimeter (Eijkelkamp Soil & Water, Giesbeek, The Netherlands), based on the International Organization for Standardization protocol 10,693 (ISO, 1995). SOC content was measured using a RC612 multiphase C and hydrogen/moisture determinator (LECO Company, St Joseph, Michigan, USA). Chemical composition of the sand, crushed dolerite and crushed concrete was determined by X-ray fluorescence spectrometry using a Panalytical PW2404 wavelength-dispersive sequential X-ray spectrometer (Malvern Panalytical Ltd, Malvern, UK). Soil pH was measured by the 1:5 soil:water suspension method (ISO, 2005), using a Jenway 3,020 pH meter (Jenway, Cole-Palmer, Stone, UK).

Due to the unreplicated nature of the demonstration plots, it was not possible to test the significance of differences between treatments using standard statistical methods. For this reason, means and standard errors are presented in bar and line graphs. A T-RF was assumed to represent one species or operational taxonomic unit (OTU) and relative abundances were used to calculate standard indices of alpha diversity (Shannon-Wiener ($H'$), evenness (E) and richness (S)). Correlation tests by either Pearson’s correlation or Spearman’s rank correlation were applied to show relationships between variables, depending on data normality. CCA was performed using the vegan package in R (Oksanen et al., 2019), to find relationships between AM fungal T-RF compositional variation and environmental parameters. Forward selection was performed to find significant environmental variables with the Monte Carlo test (999 marginal permutation). Weighted average site scores were used to decrease the degrees of noise in the environmental variables (McCune, 1997).
AMF colonization and GRSP levels are not negatively affected by artificial substrates

Colonization of roots by AMF was detected in all root samples, with levels ranging from ~25% for the 70% dolerite treatment to relatively higher levels of colonization (45–50%) for the treatment with 30% concrete as well as the two 100% treatments (Figure 2a). AMF established best of all in mixtures including concrete, which may be due to the high availability of Ca in the demolition waste (16.2% total Ca), which promoted AMF colonization in plant roots. According to Navazio and Mariani (2008), Ca²⁺ plays an important role in facilitating the interaction and contacts between plants and AMF in the early stages of symbiosis.

The EE-GRSP and DE-GRSP both showed a trend towards increasing levels as more substrate was mixed.
with the sand; this was particularly evident for the DE-GRSP, which was highest for 100% dolerite or 100% concrete (Figure 2b). In addition to facilitating symbioses between plants and AMF, the Ca$^{2+}$ supplied by concrete (present in poorly crystalline, relatively unstable, artificial silicate minerals), and to a lesser extent dolerite (7.4% total Ca, present in stable natural silicate minerals), could directly affect the formation and development of the fungal cytoskeleton, hyphal tip growth, hyphal branching and the expansion of extra-radical mycelium (ERM) (Jackson & Heath, 1993). The hyphae in the ERM network will engage in GRSP production, resulting in the high contents of DE-GRSP in the 100% treatment of dolerite and concrete. Kumar et al. (2018) showed that both EE-GRSP and DE-GRSP are strongly correlated with SOC ($r > 0.8$) in a 26-year mine land reclamation study and explained that DE-GRSP contributes to increased soil structural stability and becomes part of a stable SOC pool, whereas EE-GRSP is subject to microbial decomposition as a C substrate and converted to DE-GRSP.

**SUBSTRATE IMPACTS ON SIC VIA ENHANCED DE-GRSP**

Concrete (100%) substrates were found to have the highest SIC content (Figure 2c). Renforth, Manning, and Lopez-Capel (2009), Washbourne et al. (2012) and Manning and Renforth (2013), have all reported high SIC storage capacity in soils developed on crushed concrete. There may be some addition of lithogenic SIC in concrete demolition waste from the incorporation of calcite-containing limestone (generally up to 5% calcite in concrete) and concrete carbonation during the demolition process (Matschei, Lothenbach, & Glasser, 2007; Washbourne et al., 2012). However, the magnitude of the increase in SIC between the 70% and 100% concrete treatments suggests that there is enhancement of C capture processes producing SIC. This is supported by results of our correlation analysis that show the DE-GRSP ratio significantly positively correlated to SIC levels ($r = 0.50, p = .0083$; Table S2). We hypothesize that as more EE-GRSP decomposed to DE-GRSP (leading to an increasing DE-GRSP proportion), SIC was also increased. This is a novel discovery, because most studies have focused on positive contributions of GRSP only to SOC contents. We reason that AMF initially release EE-GRSP from their hyphae, but the high pH in concrete-based silicate soils hastens GRSP decomposition by rhizobacteria that are active in alkaline soils (Lima, Soares, & Sousa, 2013). Chemically labile EE-GRSP is weathered to DE-GRSP, and some CO$$_2$$ is released to the soil matrix (Torn, Swanston, Castanha, & Trumbore, 2009). However, in alkaline environments in concrete-based silicate soil, cations such as Ca$^{2+}$ and Mg$^{2+}$ react with this emitted CO$$_2$$ and precipitate as SIC (Gao, Tian, Pang, & Liu, 2017). The reaction appears to be more dominant in the concrete group than it is in the dolerite group. The strong degree of correlation between SIC and soil pH ($r = 0.8, p < .0001$; Table S2) indicates that the high pH in the 100% concrete (average pH = 8.4) encourages GRSP decomposition and SIC sequestration more than in the 100% dolerite (average pH = 7.5). Silicate soils show the potential to reduce C emissions to the atmosphere from GRSP turnover by precipitating the lost C in a form of SIC. Manning et al. (2013) reported that 40% of SIC in their study originated from biogenic SOC, supporting our conclusion that the C lost during the decomposition of organic C or GRSP can be sequestered as SIC.

**AMF COMMUNITY STRUCTURE LINKED TO DE-GRSP**

The CCA 1 and CCA 2 axes explained 33% of the variation of AM fungal T-RFs in soils (CCA 1, $p = .001$; CCA 2, $p = .039$) (Figure 3a). There appeared to be a distinct community of AM fungi represented by T-RFs with 57, 66, 69, 77, 79, 87, 106, 112, 148, 191, 195, 199, 205, 216 and 239 base pairs (bp) that were closely related to DE-GRSP and SOC and assumed to be high GRSP producers. These high GRSP producers were more abundant in treatments that consisted of 100% dolerite and 100% concrete than in sand or the 30, 50 and 70% mixed treatments of both dolerite and concrete. In contrast, T-RFs in the upper quadrants (52, 61, 73, 82, 92, 102, 117, 121, 123, 127, 130, 135, 144, 151, 154, 169, 173, 186, 228, 232 and 242 bp) were associated with lower-than-average values of DE-GRSP and may be lower GRSP-producing species. They were more abundant in sand and the mixed treatments of dolerite or concrete.

The first and second CCA axes in the root CCA explained 26% of the variation of AM fungal T-RFs in the roots of red fescue (CCA 1, $p = .013$; CCA 2, $p = .057$) (Figure 3b). The overall distribution of root AM fungal T-RFs was correlated with contents of DE-GRSP and SOC ($p = .011, p = .063$), but unlike the bulk soil, these T-RFs were present at an almost comparable abundance in the roots of red fescue in the 30, 50 and 70% mixed treatments or the 100% treatments of dolerite and concrete, as evidenced by the grouping of most samples in the two lower quadrants adjacent to the environmental vectors for DE-GRSP and SOC.

A few OTUs (X191, X66, X69) were located in the top right quadrant of the root CCA plot, indicating that these species were not associated with high levels of DE-GRSP or SOC when identified in the red fescue roots, in contrast to the bulk soil. Soil AM fungal DNA generally consists of fungal spores and extraradical hyphae in the ERM network that more directly engage
in GRSP production than root AM fungal structures, so species that do not appear to be dominant in the roots, can still contribute to the ERM and impact on SOC and DE-GRSP (Hempel, Renker, & Buscot, 2007). Differences in capacity for GRSP production among AMF species have also been reported in other studies, such as Campos et al. (2013), Lovelock, Wright, and Nichols (2004) and Wright, Franke-Snyder, Morton, and Upadhyaya (1996). Relatively high diversity of the AM fungal community was found in the 100% concrete, 100% dolerite and sand in both the soil and roots (Table S3) but this did not reflect any clear patterns in root colonization (Figure 2a). Diversity itself does not appear to be a direct determinant of AM fungal GRSP production. King (2011) and Rillig (2004) pointed out the importance of high GRSP producers among AMF communities in soils, rather than AMF diversity indices themselves, as certain species of AMF may lead to more GRSP production than other species in an AMF community. It is also important to keep in mind that the DNA extracted from the bulk soil reflects AMF species that associate with a diversity of plant species, whereas we only extracted DNA from the roots of one of those species (red fescue). Red fescue grows in dense tufts or clumps, so the AMF species in the bulk soil in proximity to its roots would primarily be those associated with red fescue. Nonetheless, it is still possible that some species of AMF associated with other plant species were present in the bulk soil, leading to the differences in the community structures of the AMF in the roots and soil.

To conclude, we found that crushed dolerite and concrete can not only be good substrates for recruiting high GRSP-producing groups of AMF for SOC production, but may also enhance biological processes that increase SIC sequestration. We have demonstrated that there is good potential to use recycled concrete wastes or newly crushed dolerite/basalt products for enhanced carbon sequestration as plant-growing substrates via facilitation of AMF development and GRSP production. This approach has potential not only to offset GHG emissions, but also to support biodiversity through supporting species-rich plant communities.

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AUTHOR CONTRIBUTIONS

Yejin Son: Formal analysis; investigation; visualization; writing-original draft; writing-review and editing. Kevin Stott: Conceptualization; methodology; supervision; writing-original draft. David Manning: Conceptualization; funding acquisition; methodology; project administration; supervision; writing-original draft; writing-review and editing. Julia Cooper: Formal analysis; methodology; supervision; writing-original draft; writing-review and editing. Stott: Conceptualization; methodology; supervision; writing-original draft; writing-review and editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Figshare at https://doi.org/10.6084/m9.figshare.10275935.v8

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