Urban soil microbial community and microbial-related carbon storage are severely limited by sealing

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Abstract

Purpose Urbanisation causes changes in land use, from natural or rural to urban, leading to the sealing of soil and the replacement of vegetation by buildings, roads and pavements. The sealing process impacts soil properties and services and can lead to negative consequences for microbial attributes and processes in soil. At present, information about the microbial community following soil sealing is limited. As such, we investigated how changes in soil physical and chemical properties caused by sealing affect the soil microbial community and soil ecosystem services.

Material and methods Soils were sampled beneath impervious pavements (sealed) and from adjacent pervious greenspace areas (unsealed). Soil properties (total C, total N, C:N ratio and water content) and microbial attributes (microbial biomass C, N-mineralisation and phospholipid fatty acids – PLFA) were measured and correlated.

Results and discussion A reduction of total C, total N and water content were observed in sealed soil, while the C:N ratio increased. Sealed soil also presented a reduction in microbial attributes, with low N-mineralisation revealing suppressed microbial activity. PLFA data presented positive correlations with total C, total N and water content, suggesting that the microbial community may be reduced in sealed soil as a response to soil properties. Furthermore, fungal:bacterial and gram-positive:gram-negative bacterial ratios were lower in sealed soil indicating degradation in C sequestration and a consequential effect on C storage.

Conclusions Sealing causes notable changes in soil properties leading to subsequent impacts upon the microbial community and the reduction of microbial activity and soil C storage potential.

Keywords

Urban soil, Soil sealing, Impervious surfaces, Microbial biomass, N-mineralization, PLFA, Soil carbon, Carbon storage

Declarations

Funding

This study was funded by The Faculty of Science and Technology, Lancaster University, UK.

The author Roisin O'Riordan was also supported by the Soil-Value project funded by the UKRI Engineering and Physical Sciences Research Council, Living With Environmental Change Challenge Fellowships (grant number EP/N030532/1).

Conflicts of interest/Competing interests (include appropriate disclosures)

The authors have no financial or proprietary interests in any material discussed in this article.

Ethics approval (include appropriate approvals or waivers)

Not applicable.

Consent to participate (include appropriate statements)

The authors have consented to participate in this article.

Consent for publication (include appropriate statements)

The authors have no financial or proprietary interests in any material discussed in this article and consent to publish the manuscript.

Availability of data and material (data transparency)

All authors ensure that all data and materials as well as software application or custom code support their published claims and comply with field standards.

Code availability (software application or custom code)

All authors ensure that software application or custom code support their published claims and comply with field standards.

Authors' contributions (optional: please review the submission guidelines from the journal whether statements are mandatory)

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Carly Stevens and Marlon Correa Pereira. The final draft of the manuscript was written by authors Marlon Correa Pereira and Roisin O'Riordan and all authors contributed to previous versions of the manuscript. All authors read and approved the final manuscript.

1 Introduction

Urbanisation causes considerable impacts on soil properties and services (Yan et al. 2015, 2016). Changes in land use from natural and rural to urban are associated with the replacement of vegetation by buildings, roads and pavements (Edmondson et al. 2012, 2015; Yan et al. 2016). The high degree of impermeable surfaces in cities has many negative consequences for the environment and the services it provides, particularly those provided by soil (Morgenroth et al. 2013; Wei et al. 2013; Piotrowska and Charzynski 2015; Ziter and Turner 2018; Kelleher et al. 2020).

Carbon (C) storage is an important ecosystem service provide by soil in urban areas, with vegetation biomass inputs and soil organic carbon (SOC) being key components of overall C storage (Edmondson et al. 2012; Ziter and Turner 2018). Soil sealing due to urbanisation leads to the removal of plants and topsoil during the paving and construction process. This results not only in large losses of C stocks from urban soil (Wei et al. 2014), but also alters soil C dynamics, typically leading to a loss of SOC (Majidzadeh et al. 2018). Previous soil C inventories suggested urban soil provides very little or no soil C storage (Bradley et al. 2005). However, more recently, significant amounts of soil C have been reported in urban areas, in soils of greenspaces and beneath sealed surfaces of pavements and houses (Edmondson et al 2012; Wei et al. 2014, Majidzadeh et al. 2017, Yan et al. 2016, Hu et al. 2018, Vasenev et al. 2018). As such, urban soil C and the dynamics of C storage are receiving increasing attention in research literature.

Many other key ecosystem services and soil properties are affected by soil sealing. Water infiltration is prevented or reduced, changing surface runoff patterns and seasonal dynamics of soil water content (Majidzadeh et al. 2018; Hu et al. 2020; Kelleher et al. 2020). Paving materials can act as a reservoir for contaminants such as heavy metals (Hu et al. 2018) and polycyclic aromatic hydrocarbons (Li et al. 2020); and soil temperatures can be increased (Chen et al. 2016; 2017). Gas exchange between the soil and atmosphere is reduced which can lead to higher CO_2 concentrations in sealed soil and increased CO_2 flux rate near pavement edges (Wu et al. 2016; Fini et al. 2017). Additionally, soil nutrient content can be altered, with sealed soils exhibiting increased calcium, potassium, sodium and phosphorous; and decreased aluminium, iron, magnesium and nitrogen (Zhao et al. 2012; Morgenroth et al. 2013; Hu et al. 2018; Majidzadeh et al. 2018). The severe decrease in nitrogen (N) can lead to very high CN ratios in sealed soils, despite the concurrent loss of soil C (Zhao et al. 2012, Hu et al. 2018).

These changes to the soil environment also affect soil microbes, which may impact the microbial processes and activities that underpin many important soil services (Zhao et al. 2012). Whilst sealed soils remain largely understudied, a small number of studies have observed that sealing can lead to a decrease in microbial biomass C, microbial biomass N, enzyme activities and respiration potential (Zhao et al. 2012, Wei et al. 2013, Piotrowska and Charzynski 2015), as well as a decrease in N-mineralisation potential (Zhao et al. 2012; Majidzadeh et al. 2018). Similarly, sealing has led to changes in bacterial communities, with a reduction in alpha diversity and a distinct community found in sealed soil when compared with unsealed soil (Hu et al. 2018, Yu et al. 2019). Research has shown that sealing has a negative effect on urban soil microbial attributes and bacterial communities, although little is known about the dynamics of both bacterial and fungal communities and their contribution to the soil microbial community in sealed soils. Furthermore, there is a gap in knowledge into what these altered bacterial and fungal dynamics mean for important soil ecosystem services such as nutrient cycling and C storage within sealed soils. Fungal:bacterial dominance is considered an important factor in C sequestration (Strickland and Rousk 2010); and the ratio between gram positive:gram negative bacteria provides insight into the stability or recalcitrance of C in the soil (Fanin et al. 2019). At present these dynamics have not been studied in sealed soil, and therefore the implications for soil C storage across the urban landscape are currently unknown.

In this paper we investigate how changes to soil physical and chemical properties caused by sealing affect the microbial community and microbial attributes. The city of Lancaster (UK) and surrounding urban areas were used as a study site. We measure soil properties (total C, total N, C:N ratio and water content) and microbial attributes (microbial biomass C, phospholipid fatty acids and N-mineralisation) to make a comparison across sealed and unsealed soils. To our knowledge, we present the first investigation into bacterial and fungal dynamics in sealed soil using phospholipid fatty acid analysis, and consider their contributions to the soil microbial community and consequences for important soil services. We hypothesise that, (i) sealing leads to large changes in soil properties; and (ii) sealing leads to changes in microbial attributes, significantly altering community composition and reducing microbial activity. Measurements of soil total C, total N, C:N ratio and water content provided indicators of the impacts of sealing on soil properties (hypothesis 1). Microbial biomass C, phospholipid fatty acids and N-mineralisation were used as indicators of changes in microbial attributes, with biomass C and phospholipid fatty acids pointing to changes in community composition; and N-mineralisation to changes in microbial activity (hypothesis 2).

2 Materials and methods

2.1 Study area

The study area consisted of the medium-sized UK city of Lancaster and the surrounding urban areas (Fig. 1). The National Soil Map for England and Wales, accessed on the Soilscapes viewer online (Cranfield, 2020), shows that across much of Lancaster city there are freely draining slightly acid loamy soils, while sampling sites in the surrounding areas tended to be on slowly permeable seasonally wet acid loamy and clayey soils.

2.2 Soil sampling

Sealed soils were collected from 25 roadworks sites where works had exposed the soil beneath pavements and roads. Sealing had occurred at different times in the past, and further research is still needed to determine if the time since sealing has an impact on the measured variables. Soil was collected from the top 10 cm of soil below the sealed surface and human-made layers. To allow a comparison between soils, an unsealed sample was collected from the nearest available greenspace after each sealed soil was collected. Unsealed samples were collected from the top 10 cm of soil, primarily from grass covered road verges, amenity greenspaces and residential gardens, with a distance ranging from 0.5 to 15 m of the respective sealed site. Approximately 500 g of both soils (50 samples) were collected with a trowel and were immediately returned to the lab for refrigeration prior to fresh soil tests.

2.3 Soil preparation and analysis

Soil properties and CN analysis

Soil water content was determined gravimetrically by drying the samples at 105 °C for 24 hours. The dried sample was ball milled to a powder and analysed for total C and total N using a dry combustion CN analyzer (Vario Max CN).

Microbial biomass C and N-mineralisation

Microbial biomass C (MBC) was determined using the chloroform fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987). Two subsamples of 5 g of moisture adjusted soil were prepared for each sample, one fumigated with alcohol-free $CHCl_3$ for 24 hours; and one non-fumigated stored at 4 °C. After removal of the $CHCl_3$, both subsamples were extracted with 25 mL of K_2SO_4 (0.5 M) for 30 minutes. The filtrate was analyzed for extracted C using a TOC analyzer (Shimadzu TOC-L_{CPN} TN).

Soil potential N-mineralisation was measured before and after incubation. Subsamples were prepared for water saturation to determine moisture adjustments for each sample. The subsamples were placed in a funnel with Whatman nº 1 filter paper, wet with MilliQ water, and periodically re-wet over a 2-hour period. They were then covered with cling film and drained for 2 hours, weighed, and oven dried at 105 °C for 24 hours. They were re-weighed and moisture adjustments were calculated to 60 % for each sample. For extractions, 5 g of moisture adjusted soil was put in an extraction bottle, covered with covered with polythene and incubated at 25 °C for 14 days. A second sample was extracted immediately. The incubated and non-incubated subsamples were extracted using KCl (1 M), and the filtrate was analysed for inorganic N using an auto-analyzer (Elementar Vario EL III). Potential N-mineralisation was calculated as the difference in inorganic N before and after incubation.

Phospholipid fatty acid analysis

Phospholipid fatty acid (PLFA) analysis was used to determine the overall microbial community composition and dominance. Soil subsamples were taken from soils previously stored at − 80 °C and extracted for PLFA determination by gas chromatography (Vestle and White 1989; Willers et al. 2015). Microbial PLFA markers were identified and measured as per the method by Frostegård et al. (2011) to estimate the total and group-specific microbial marker biomass. The i15:0, a15:0, i16:0, a17:0 and i17:0 PLFA markers were used as gram positive (GP) bacteria markers; and 16:1ω7, cy17:0, cis18:1ω7 and cy19:0 as gram negative (GN) bacteria markers. Total bacteria were estimated from the sum of GP and GN bacteria, and 15:0 marker mass. Total fungi were measured using 18:1ω9 and 18:2ω6,9 as markers. The 16:1ω5 was used as a proxy measurement for arbuscular mycorrhizal (AM) fungi. Total PLFA expresses total microbial marker biomass and was estimated as the sum of total bacteria, total fungi, AM fungi and 16:0, 16:1ω7, br17:0, 17:1ω8, 17:0 7-methyl, 18:0, br18:0, 18:1ω5 and 19:1 markers. The fungal:bacterial and GP:GN ratios were calculated by dividing the respective biomarker masses.

2.4 Statistical analysis

Data were evaluated using R (version 4.0) on the software RStudio (version 1.1.463). Since only water content and total C in unsealed soil presented data with a normal distribution according to the Shapiro-Wilk test, the non-parametric Wilcoxon test was applied. Where microbial attributes presented values equal to zero they were considered null values (Table 1); while some soil samples did not present detectable amounts of PLFA during gas chromatography and so were excluded from the analysis. Boxplots were constructed using the *ggplot* package and statistical significance was presented to compare sealed and unsealed soils. The correlations between soil properties and microbial attributes were estimated using the Spearman's rank correlation (*ggcorrplot* package).

3 Results

Sealed soils exhibited consistently lower values than unsealed soils across all measured soil properties and microbial attributes, other than the C:N ratio (Table 1). Total C ($p = 0.0026$), total N ($p < 0.001$), and water content ($p < 0.001$) were all significantly lower in sealed soil than unsealed soil (Fig. 2A, B and D), while the C:N ratio ($p = 0.023$) was higher in sealed soil (Fig. 2C). All microbial attributes exhibited significantly lower values in sealed soil than unsealed soil: MBC, N-mineralisation, total PLFA, total fungi, AM fungi, total bacteria, GP bacteria and GN bacteria presented $p < 0.001$; fungal:bacterial ratio presented $p = 0.019$; and GP:GN bacterial ratio presented $p = 0.0017$ (Fig. 3 and Fig. 4).

Significant correlations were observed between soil properties and microbial PLFA attributes, however, MBC and N-mineralisation potential showed no correlation with soil properties in this study (Table 2). In sealed soil, total bacteria had a strong and positive correlation with total N (rho = 0.63, $p = 0.038$) and water content (rho = 0.71, $p = 0.015$); GP bacteria a strong and positive correlation with total N (rho = 0.63, $p = 0.038$) and water content (rho = 0.71, $p = 0.015$); and GN bacteria a strong and positive correlation with total C (rho = 0.64, $p = 0.032$), total N (rho = 0.71, $p = 0.015$) and water content (rho = 0.79, $p = 0.004$). In unsealed soil, total PLFA, total fungi, total bacteria and GP bacteria presented moderate to strong positive correlations with total C (rho = 0.58, $p = 0.020$; rho = 0.59, $p = 0.019$; rho = 0.56, $p = 0.025$ and rho = 0.52, $p = 0.042$, respectively); total N (rho = 0.62, $p = 0.012$; rho = 0.54, $p = 0.034$; rho = 0.68, $p = 0.005$; and rho = 0.69, $p = 0.004$, respectively); and water content (rho = 0.75, $p = 0.001$; rho = 0.75, $p = 0.001$; rho = 0.68, $p = 0.005$; and rho = 0.66, $p = 0.007$, respectively). GN bacteria had a strong positive correlation with total N (rho = 0.61, $p = 0.015$) and water content (rho = 0.65, $p = 0.008$); and the GP:GN bacterial ratio showed a moderate positive correlation with total C (rho = 0.52, $p = 0.040$).

4 Discussion

In contrasting soil samples from sealed and unsealed areas, we observed that sealing affects soil properties, reduces the microbial community and limits microbial processes; changes which may disrupt important soil ecosystem services. Soil properties were notably altered in sealed areas, with a reduction of total C, total N and water content, and a consequent increase in C:N ratio. Sealing had a negative impact on microbial attributes, with a large reduction of the microbial community (MBC and PLFA biomarkers) and activity (N-mineralisation). Additionally, microbial attributes that correlated with soil properties in unsealed soil did not show equivalent correlations in sealed soil, such as those between total PLFA and total fungi to total C, and total N and water content. These results suggest that the microbial community in sealed soil may respond differently to that in unsealed soil, indicating that sealing may disrupt the microbial response to changes in soil properties and lead to negative impacts on microbial services. The PLFA data provides an indicator of the microbial community in sealed soil, where low fungal:bacterial and gram-positive:gram-negative bacterial ratios indicate degradation in microbial C sequestration and a consequential effect on soil C storage in sealed soil.

4.1 Soil sealing leads to depletion of C, N and water content

The sealed soils exhibited lower total C, total N and water content than unsealed soils (Table 2 and Fig. 2A). Soil sealing leads to a reduction of soil C due to topsoil removal during the construction process and the reduction of C inputs from organic matter, plant root exudates and residue decomposition (Edmondson et al. 2012; Raciti et al. 2012; Wei et al. 2013, 2014; Piotrowska and Charzynski 2015; Yan et al. 2015; Majidzadeh et al. 2017, 2018). Indeed, sealed soils have been recorded as having significantly lower C stores when compared with unsealed or greenspace soils in urban areas (Wei et al. 2014; Piotrowska-Długosz and Charzyński 2015; Majidzadeh et al. 2017). Additionally, if C decomposition continues within sealed soil, even at a low rate (Wei et al. 2014; Piotrowska and Charzynski 2015), and there are negligible C inputs (Majidzadeh et al. 2018), this will contribute to C losses. In this context, elevation of microbial C respiration in sealed soil has been linked to increases in water content (Piotrowska and Charzynski 2015; Majidzadeh et al. 2017, 2018). In sealed soil, water content is affected by the type and size of pavement or sealing surface (Morgenroth et al. 2013), and beneath impervious and semi-permeable pavements the water content is, in general, lower than in greenspace soils (Hu et al. 2018; Piotrowska and Charzynski 2015). In soil under semi-permeable surfaces, water moving from adjacent greenspaces into sealed soil can promote C inputs beneath sealed surfaces (Majidzadeh et al. 2018); however, this can also increase the microbial processes of C decomposition and lead to C losses (Majidzadeh et al. 2017, 2018). In soil under house crawl spaces of different ages, most C was lost in the first 50 years after construction, but after 50 years, C sequestration became the dominant process (Majidzadeh et al. 2018). Overall, it is not clear whether longer periods of sealing lead to an increase or decrease in the C balance of sealed soils, and this is an area which requires further investigation.

The notable depletion of total N, as seen in our results (Fig. 2B), is a commonly observed consequence of soil sealing, often being greater in magnitude than losses of total or organic C (Raciti et al. 2012; Zhao et al. 2012; Wei et al. 2014; Majidzadeh et al. 2018; Hu et al. 2018). Our results indicate that in sealed soil total N was reduced by over 60 % compared to unsealed soil (Fig. 2B); while total C was reduced by nearly 40 % compared to unsealed soil (Fig. 2A), leading to a higher C:N ratio in sealed soil (Fig. 2C). Our results are comparable to other observations of sealed soil where total C reduction was between 42 and 57 %; and N depletion was between 47 and 97 % (Majidzadeh et al. 2018; Piotrowska et al. 2015; Raciti et al. 2012; Zhao et al. 2012). The effect of sealing appears to be most notable and variable for N dynamics and processes, which can be connected to the length of time sealed, organic C availability and water content; influencing the sealing impact on microbial processes (Zhao et al. 2012; Piotrowska et al. 2015; Majidzadeh et al. 2017, 2018) and N-mineralisation potential (Fig. 3B, Zhao et al. 2012). Previous research has shown that sufficient water content can promote microbial decomposition and N-mineralisation where there is available organic C (Zhao et al. 2012; Majidzadeh et al. 2018), leading to inorganic N production (Zhao et al. 2012; Majidzadeh et al. 2018), and potential leaching of NH_4^+-N and NO_3^--N and accumulation in the sub-soil (Zhao et al. 2012). Where water can infiltrate into sealed soils from adjacent unsealed areas (Majidzadeh et al. 2018), we speculate that mineralization of remaining organic matter could be stimulated. Considering, the reduced levels of C and the absence of plant roots, N assimilation by microorganisms and plants is likely to be low, resulting in N losses over time by leaching, subsoil accumulation and groundwater transport. Beyond that, these circumstances may lead to inorganic N pollution of urban groundwater and water courses (Zhao et al. 2012).

4.2 Sealing alters microbial attributes and community composition

Soil sealing leads to a drastic reduction in microbial attributes. Our results showed that sealed soil exhibited a reduction in MBC (Fig. 3A), as consistently reported in previous studies (Wei et al. 2013; Piotrowska and Charzynski 2015; Majidzadeh et al. 2017, 2018). Observations of low MBC in sealed soil have commonly been associated with low C, N and water content (Wei et al. 2013; Piotrowska and Charzynski 2015; Majidzadeh et al. 2017, 2018; Hu et al. 2018). Our PLFA data also demonstrated the negative impact of sealing on the microbial community (Fig. 3), with sealed soil exhibiting significantly lower mass of total PLFA and microbial markers, consistent with reductions in MBC, total C, total N and water content. It has been observed that a reduction in the microbial community reflects low microbial activity (Zhao et al. 2012; Piotrowska and Charzynski 2015), a pattern also observed in our results with the significantly reduced N-mineralisation potential in sealed soil.

In studies of urban soil, few have considered the relationship between soil properties and microbial attributes in both sealed and unsealed soil. Indeed, physical and chemical properties, in particular water content, have been shown to have significant effects on microbial attributes in unsealed soils (Wei et al. 2014; Piotrowska and Charzynski 2015); and have exhibited positive correlations with MBC, catalase activity and β-glucosidase activity in unsealed soil, but not in sealed soil (Piotrowska and Charzynski 2015). Here, neither MBC nor N-mineralisation potential had significant correlations with any soil properties across sealed or unsealed soils. Conversely, the PLFA data does show significant responses of the microbial community to soil properties (Table 2). In unsealed soil, increases in C, N and water content correlated with growth of the microbial community (total PLFA, bacteria and fungi), which is typical for natural soils or those under agricultural conservation management (Helgason et al. 2014; Bai et al. 2020). However, in sealed soil, only bacteria correlated with soil properties, suggesting that sealing disrupts the relationships normally seen in natural and agricultural soils between microbial attributes and soil properties. And the importance of total N and water content could be highlighted from our data, once both affected positively total, GP and GN bacteria of sealed and unsealed soil (Table 2), indicating that input of water and N promoted bacterial growth. Other studies have found additional soil properties associated with sealing-driven microbial depletion, including potassium and phosphorus availability, heavy metals and dissolved organic C (Hu et al. 2018; Yu et al. 2019). Low respiration and metabolic quotient observed on sealed soil (Piotrowska and Charzynski 2015) can still suggest organic matter of low quality. Thus, sealing results in alterations to soil properties and negative impacts on the soil microbial community and processes.

Sealing also caused alterations to the microbial community composition, notably the fungal:bacterial ratio and GP:GN ratio. The effect of sealing was seen more strongly in fungi, with sealed soils having ~ 93 % less fungi than unsealed soils, and ~ 78 % less bacteria than unsealed soils. Consequently, the fungal:bacterial ratio decreased in sealed soils indicating greater numbers of bacteria to fungi (Fig. 4G). Fungi have been shown to be resistant to conditions of low total N, high C:N ratio and low water content (Six et al. 2006; Strickland and Rousk 2010; Fang et al. 2020); conditions which are commonly observed in sealed soils. However, these conditions did not lead to greater dominance of fungi in this study. Conversely, soils affected by degradation processes such as tillage, deforestation, trampling and contamination usually present a greater impact on the fungal community and show a proportional decrease on the fungal:bacterial ratio (Kaur et al. 2005; Malmivaara-Lämsä et al. 2008; Simmons and Coleman 2008; Bischoff et al. 2016; Montiel-Rozas et el. 2018; Lopes and

Fernandes 2020). Thus, our results suggest that fungi in sealed soils may be more affected by aspects of soil sealing not included in this study but that commonly arise due to the degradation processes of urbanization, such as contamination and disturbance.

The decrease in the GP:GN bacterial ratio in sealed soil (Fig. 4H) suggests that GN bacteria are more adapted to sealing than GP bacteria. GN bacteria presented a positive correlation with total C, while GP bacteria had no correlation with total C (Table 2). As GN bacteria are more dependent on simple sugars (Kramer and Gleixner 2008; Fanin et al. 2019), the organic C that is promoting GN bacterial growth is likely to be labile and soluble C transported by water from adjacent greenspaces, a process which has been suggested as a source of organic C in soils beneath house crawl spaces (Majidzadeh et al. 2018). Additionally, GN and GP bacteria had positive correlations with total N and water content, suggesting there may also be transport of soluble N by water from adjacent greenspaces, and that this may be an important source of nutrients for bacteria in sealed soil.

In contrast to GN bacteria, GP bacteria are linked to more complex SOC (Kramer and Gleixner 2008; Fanin et al. 2019). Therefore, the low biomass of GP bacteria can be related to low levels of complex SOC remaining in sealed soil as a consequence of topsoil removal and microbial degradation over time.

4.3 Sealing limits the microbial community and affects the C storage service

Litter degradation plays an important role in C inputs into soil. Organic and inorganic compounds released during decomposition and the remaining complex organic compounds are essential components of soil organic matter synthesis (Jastrow et al. 2007). In sealed soil, the sealed surface acts as a barrier preventing this source of organic C from reaching the soil, such that low or no organic C or nutrients from litter can enter the soil (Zhao et al. 2012; Majidzadeh et al. 2017, 2018), which in turn, affects soil biological and nutrient processes.

Plants and roots also contribute greatly to soil C stores. The lack of plants growing on sealed surfaces usually leads to a reduced root colonization, limiting the C inputs from plant exudates and dead roots. Consequently, microbial processes that take place in the soil-root zone and depend on plant exudates are limited beneath sealed surfaces. Many of these processes are related to N inputs and nutrient availability, highlighting N biological fixation, N oxidation reactions and phosphate solubility (Sylvia et al. 2005; Paul 2007). Many fungal species establish a mutualistic association with plant roots to obtain organic molecules and, as payment, they colonize soil space to assimilate and transport nutrients directly back to the plant roots (Smith and Read 2008). By enhancing the soil microbial community, roots enable microbial processes connected with organic matter formation, such as the microbial release of biomolecules and dead biomass (Jastrow et al. 2007; Clemmensen et al. 2013). Thus, it is likely that the lack of plant and root growth, litter inputs and microbial activity in the soil-root zone all contribute to the lower C stores in sealed soil.

Fungal biomass in soil is, in general, suggested to contribute to high soil C storage (Strickland and Rousk 2010). Fungi exhibit low nutrient requirements and high C use efficiency which results in more C being allocated to their biomass, per unit of substrate used, compared to bacteria, which have lower C use efficiency (Six et al. 2006). Fungi have the ability to grow under a high C:N ratio, permitting their mycelial growth to explore wider areas and translocate nutrients across the soil (Strickland and Rousk 2010). In addition, fungal biomass is more complex and resistant to decomposition than bacterial biomass, introducing a more stable form of organic C in the soil (Jastrow et al. 2007; Clemmensen et al. 2013). While studies have presented different insights into the functional implications of the fungal:bacterial ratio (Strickland and Rousk 2010; Soares and Rousk 2019), in general, a higher fungal:bacterial ratio is assumed to promote an increase in soil organic matter (Jastrow et al. 2007; Strickland and Rousk 2010). Therefore, the observed reduction in fungi and consequent bacterial dominance in sealed soil is likely to lead to notable limitations to C storage.

The lower GP:GN bacteria ratio in sealed soil illustrates that there is more GN bacteria to GP. This indicates that there is less recalcitrant C in the sealed soil (Kramer and Gleixner 2008; Fanin et al. 2019), which suggests the reduced ability of sealed soils not only to store C, but to store it as stable C that may be more protected from decomposition (Lal 2004, Marschner et al. 2008), highlighting the wider impacts of soil sealing on the ecosystem service of soil C storage.

5 Conclusion

Soil properties were notably affected in sealed soil, with a large significant reduction in total C, total N and water content in sealed soils. Microbial biomass C, N-mineralisation potential and microbial PLFA markers were also significantly reduced in sealed soils. Our results show that changes to soil properties, caused by sealing, led to a drastic decrease in the microbial community and important microbial processes. The increase of the C:N ratio and decrease of the F:B and GP:GN ratios suggest that sealed soils are degraded due to the loss of C, which limits fungal and bacterial growth. In addition, the reduced inputs of C from litter degradation and plant exudates, associated with the reduction of fungal dominance, indicate a limitation on the C storage potential of sealed soil. Furthermore, the correlation of bacteria with C, N and water suggests there may transport of soluble C and N by water into sealed soils from adjacent greenspaces. This may be an important source of nutrients for microbes in sealed soil, and the investigation of this process would be beneficial to further understand sealed soil nutrient cycling and implications for C and N fluxes. In this context, further work, such chronosequence studies, would elucidate how urbanisation and soil sealing impact the dynamics of C and N and microbial processes over time, and as a consequence, the ecosystem services of sealed soil.

ACKNOWLEDGEMENTS

This study was funded by Lancaster University Faculty of Science and Technology and the UKRI Engineering and Physical Sciences Research Council (grant number EP/N030532/1).

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TABLE

Table 1 Descriptive statistics of soil properties and microbial attributes in sealed and unsealed soils

Variable groups	**Variables**	**Pavement types**	**n***	**Null***	**Min – Max***	**Mean ± SE***	**CV* (%)**
Soil properties	Total C g/Kg	Sealed	25	0	3.35 - 250.29	49.78 ± 10.67	107.12
		Unsealed	25	0	14.02 - 128.49	73.49 ± 5.33	36.27
	Total N g/Kg	Sealed	25	0	0.39 - 13.75	2.08 ± 0.587	141.03
		Unsealed	25	0	0.026 - 21.75	5.36 ± 0.79	73.24
	C:N ratio	Sealed	25	0	4.92 - 149.87	35.81 ± 7.13	99.57
		Unsealed	25	0	5.91 - 27.49	15.55 ± 1.11	35.62
	Water content g/g	Sealed	25	0	0.09 - 0.74	0.30 ± 0.03	54.09
		Unsealed	25	0	0.08 - 0.85	0.47 ± 0.03	32.83
Microbial attributes	MBC g/Kg	Sealed	25	7	0 - 47.85	6.11 ± 2.17	177.67
		Unsealed	25	0	1.99 - 58.59	19.79 ± 3.04	76.69
	Mineralization g/Kg	Sealed	25	12	0 - 2.87	0.42 ± 0.15	178.14
		Unsealed	25	1	0 - 21.22	5.61 ± 1.03	92.12
	Total PLFA mg/Kg	Sealed	11	0	0.007 - 2.176	0.311 ± 0.198	211.30
		Unsealed	16	0	0.338 - 2.996	1.101 ± 0.164	59.61
	Fungi mg/Kg	Sealed	11	3	0 - 0.239	0.036 ± 0.021	197.03
		Unsealed	16	0	0.118 - 0.867	0.357 ± 0.050	56.33
	AM fungi mg/Kg	Sealed	11	8	0 - 0.019	0.003 ± 0.002	230.69
		Unsealed	16	0	0.008 - 0.146	0.062 ± 0.009	60.98
	Bacteria mg/Kg	Sealed	11	5	0 - 0.832	0.094 ± 0.075	263.19
		Unsealed	16	0	0.075 - 0.821	0.304 ± 0.045	58.83
	GP bacteria mg/Kg	Sealed	11	5	0 - 0.364	0.043 ± 0.033	249.92
		Unsealed	16	0	0.044 - 0.572	0.187 ± 0.032	68.37
	GN bacteria mg/Kg	Sealed	11	6	0 - 0.468	0.050 ± 0.042	277.50
		Unsealed	16	0	0.031 - 0.236	0.113 ± 0.013	45.08
	Fungal:Bacterial ratio	Sealed	10	4	0 - 2.470	0.663 ± 0.284	135.20
		Unsealed	16	0	0.717 - 1.585	1.206 ± 0.062	20.57
	GP:GN bacterial ratio	Sealed	10	5	0 - 2.151	0.628 ± 0.237	119.30
		Unsealed	16	0	0.958 - 2.428	1.584 ± 0.104	26.16

*n: the number of values; null: the number of null values; min: the minimal value; max: the maximal value; SE: the standard error of the mean; CV: the coefficient of variation.

Table 2 Spearman's rank correlation (rho) and p-values of correlations between microbial attributes and soil properties in sealed and unsealed soils. Significant correlations with p-values < 0.05 are indicated in bold.

Microbial attribute	**Soil status**	**Total C**		**Total N**		**C:N ratio**		**Water content**	
		rho	p-value	rho	p-value	rho	p-value	rho	p-value
MBC	Sealed	0.31	0.356	0.61	0.052	-0.27	0.418	0.47	0.146
	Unsealed	0.50	0.051	0.20	0.450	0.35	0.188	0.41	0.114
N-mineralisation potential	Sealed	-0.04	0.902	-0.21	0.534	0.02	0.951	-0.18	0.598
	Unsealed	0.29	0.278	0.25	0.343	0.04	0.891	-0.04	0.891
Total PLFA	Sealed	0.57	0.071	0.55	0.082	0.13	0.714	0.55	0.087
	Unsealed	0.58	**0.020**	0.62	**0.012**	-0.08	0.771	0.75	**0.001**
Total fungi	Sealed	0.46	0.156	0.5	0.113	0.03	0.936	0.5	0.121
	Unsealed	0.59	**0.019**	0.54	**0.034**	-0.01	0.978	0.75	**0.001**
Total bacteria	Sealed	0.56	0.072	0.63	**0.038**	-0.13	0.696	0.71	**0.015**
	Unsealed	0.56	**0.025**	0.68	**0.005**	-0.13	0.633	0.68	**0.005**
Fungal:Bacterial ratio	Sealed	0.23	0.499	0.09	0.802	0.19	0.574	0.23	0.499
	Unsealed	0.29	0.283	-0.22	0.404	0.48	0.064	0.03	0.926
GP bacteria	Sealed	0.56	0.072	0.63	**0.038**	-0.13	0.696	0.71	**0.015**
	Unsealed	0.52	**0.042**	0.69	**0.004**	-0.19	0.484	0.66	**0.007**
GN bacteria	Sealed	0.64	**0.032**	0.71	**0.015**	-0.19	0.569	0.79	**0.004**
	Unsealed	0.21	0.443	0.61	**0.015**	-0.42	0.104	0.65	**0.008**
GP:GN bacterial ratio	Sealed	0.42	0.203	0.55	0.079	-0.19	0.569	0.68	**0.022**
	Unsealed	0.52	**0.040**	0.47	0.070	0.01	0.969	0.33	0.217

FIGURE CAPTIONS

Fig. 1 Location of sampling sites, indicated on the map with black dots.

Fig. 2 Soil properties in sealed and unsealed soils. (A)Total C, (B) total N, (C) C:N ratio and (D) water content. A significant difference between sealed and unsealed soil was estimated by Kruskal-Wallis test, with "****", "***", "**" and "*" indicating significance at p < 0.0001, p < 0.001, p < 0.01 and p < 0.05, respectively.

Fig. 3 Microbial biomass C (MBC) and N-mineralisation potential in sealed and unsealed soils. A significant difference between sealed and unsealed soil was estimated by Kruskal-Wallis test, with "****" indicating significance at p < 0.0001.

Fig. 4 Microbial community in sealed and unsealed soils. (A) total PLFA, (B) total fungi, (C) AM fungi, (D) total bacteria, (E) GP bacteria, (F) GN bacteria, (G) fungal:bacteria ratio and (H) GP:GN bacterial ratio. A significant difference between sealed and unsealed soil was estimated by Kruskal-Wallis test, with "****", "***" and "**" indicating significance at p < 0.0001, p < 0.001 and p < 0.01, respectively.