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Evolution of Physico-Chemical Properties, Microbial Biomass and Microbial Activity of an Urban Soil after De-Sealing

Giancarlo Renella [ORCID]

Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, 35020 Legnaro, Italy; giancarlo.renella@unipd.it

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Abstract: Recovery of soil fertility after de-sealing of urban soils is still poorly known. This work studied the time-related dynamics of soil physico-chemical and biochemical endpoints of urban soil in the city in Naples (Southern Italy), de-sealed for different time during construction works, that underwent colonization by volunteer plants. The results showed de-sealing decreased the soil bulk density and the soil pH value, increased the electrical conductivity (EC), total organic C (TOC) and extractable carbohydrates (TEC), total and inorganic N contents, soil basal respiration (SBR), soil microbial biomass C (MBC) and soil microbial biomass N (MBN), the substrate induced respiration (SIR) value, and enzyme activities involved in C, N, P and S mineralization. The TEC, total and inorganic N, SBR and microbial biochemical endpoints were higher in the de-sealed soils than those of an arable soil of the same area. The results show that de-sealed urban soils rapidly increase their physical, chemical and biological fertility even with no intervention, especially when they are colonized by volunteer plants.

1. Introduction

Urbanization consumes soil mainly by sealing, and is among the major causes of soil loss in the European Union and worldwide [1]. Sealing of soil by impermeable layers separate it from the atmosphere and hydrosphere [2], reduces its ecological functionality related to water infiltration [3], reduces microbial diversity and microbial activity [4] due to reduced inputs of organic matter [5] and contributes to the adverse effects on urban microclimate [6,7]. Biomass and activity of soil microorganisms depend on the quantitative and qualitative inputs of litter and organic matter, as soil microorganisms obtain energy from their decomposition and mineralization, and the impacts of sealing on soil microbially driven soil functions have been well documented [8].

According to the EU Roadmap to a resource efficient Europe [9], soil is a limited and non-renewable natural resource responsible for multiple ecosystem services, and is an integral part of the natural capital; for these reasons, the EU strategy urges all member States to increase their knowledge on the potential recovery of ecological functionality of impacted urban soils, particularly sealed soils [10]. However, compared to the impact of soil sealing, less is known about the evolution of chemical parameters and biochemical activity of de-sealed soils, which can provide information on the potential of de-sealed soils to gain fertility over time, particularly for those left with no intervention. Fertility is the ability of a soil to support a plant community providing plants with adequate porosity, nutrient levels and microbial activity, and is the soil characteristic needed for restoration interventions of urban areas. Among the soil constituents, soil organic matter (SOM) is the key component quantitatively and

qualitatively affected by soil sealing and de-sealing. The SOM plays key roles in soil as it stabilizes the soil aggregates increasing soil water-holding capacity, increases the soil buffer capacity of pH values, and is a reservoir pool of nutrients [11,12]. Therefore, the analysis of the SOM content and C pools can provide indications of the evolution of de-sealed soils. The SOM content and quality depends on the vegetation cover, with grasslands having the most stable SOM contents due to continuous litter and rhizodeposition inputs [13,14]. The SOM sustains the microbial biomass and microbial metabolic activity, and a reduction of SOM generally causes reduction of soil respiration, soil microbial biomass and soil enzyme activity [15–18]. It has been reported that microbial activity and microbial diversity in managed urban soils depends on the age and complexity of the plant communities [19,20], and for this reason, the increase of the soil microbial biomass and microbiological activities have been used biochemical endpoints of increased labile SOM inputs, and more in general of rehabilitation for soils after de-sealing. However, the knowledge of the dynamics of microbial biomass and activity in de-sealed soils, particularly in the early stages of volunteer plant colonization, is still scarce.

The objective of the study was to describe the evolution of key physical, chemical and biological properties of an urban soil de-sealed for different time, undergoing colonization by volunteer plants. I hypothesized that under the action of volunteer plants, de-sealed soils could increase their fertility endpoints to values comparable to those of an agricultural soil, even under no intervention. The results of this study can contribute to a better understanding of the potential induction of fertility in de-sealed urban soils, in the perspective of sustainable urban regeneration practices, in line with the EU Roadmap to a resource efficient Europe [9].

2. Materials and Methods

2.1. Study Area, Soil Sampling and Preparation

The studied soils are located in Eastern territory of the municipality of Naples (Southern Italy, Latitude 40.8359336, Longitude 14.2487826, 29 m a.s.l.), with Mediterranean Oceanic climate characterized by mean annual rainfall of 952 mm and average temperature of 17.5 °C. The area underwent urban sprawl beginning in the 1920's, and currently has 13% of its surface covered by roads and railways, 63% active and dismissed industries, and the remaining surface as residential or used for agriculture. The studied soils are Andosols originated on alluvial sediments and pyroclastic deposits from volcanic parent materials [21], and the area was classified as polluted by the Italian Agency for Environmental Protection due to soil and water pollution by metals and metalloids, polycyclic hydrocarbons (PAH) and polychlorinated hydrocarbons (PCB) causing high incidence of cancer in the population (Law n. 426/1998). Soil samples were taken from an approximately 2 km long × 0.1 km wide construction site where the surface asphalt layer and gravel were removed from different areas for 2 months (DS2), 20 months (DS20) and 34 (DS34) months. The DS20 and DS34 sampling areas were colonized by volunteer broadleaf plants *Amaranthus retroflexus* (L.) and *Blackstonia perfoliata* (L.). The samples were manually taken from selected rectangular areas of 2 m × 5 m for each sampling area, and 5 samples from 0 cm to 20 cm depth using an auger, resulting in 15 independent samples. The soil from a smallholder orchard located 1 km westbound from the construction site was sampled in 5 points and used for comparing the physico-chemical properties and biochemical activity of the de-sealed soils with those of a fertile soil of the same area. All soils were sampled on the same day, were immediately brought to the analytical laboratories, and each field moist individual soil sample was passed through a 2 mm sieve. Each sieved soil was then split in two portions of approximately 80 dry weight each: one part was used fresh for the analysis of soil biochemical properties and the other part was air-dried at room temperature and used for the analysis of soil chemical properties.

2.2. Analysis of Soil Chemical Properties

The particle-size distribution was determined using the hydrometer method [22]. The bulk density (BD) was determined using undisturbed soil cores for each sampling area plot taken by a stainless steel cylinder of 50 cm^3; the soil cores were saturated with distilled water, then oven-dried at 105 °C, and the difference between the water-saturated and dry soil was used to calculate the BD as the value of the dry soil weight/soil volume ratio value. Soil pH and electrical conductivity (EC) values were determined using 1:2 soil:water (*w:v*) suspension by a pH-meter and a conductometer (Metrohm, Herisau, Switzerland). The cation exchange capacity (CEC) was measured using the 1 N ammonium acetate method [23]. Total C and N were determined with a Carlo Erba C/N 1500 analyzer, the total organic carbon (TOC) was determined by wet the oxidation methods of [24]. Total extractable carbohydrates (TEC) were quantified by extraction of 1 g of soil shaken with 10 mL of 1M H_2SO_4 for 16 h according to [25]. The supernatant solutions were used to determine the carbohydrate concentration using the phenol-sulfuric acid colorimetric method of [26] by UV spectrophotometry (Lambda 2, Perkin Elmer, Waltham, MA, USA), measuring the absorbance of light at 490 nm, and using a calibration curve based on a glucose concentration range of 0–10 mM.

2.3. Measurement of Soil Respiration, Soil Microbial Biomass, Substrate Induced Respiration, and Soil Enzyme Activities

For measurement of soil basal respiration (SBR) and substrate induced respiration (SIR), the soil samples of 10 g dry weight equivalent were enclosed in 1 L air-tight jars along with a vial containing 5 mL of 0.5 M NaOH for 4 and 1 day, respectively. For the SIR determination, the soil samples were amended with 1 mL of glucose solution resulting in a 2 mg glucose-C g^{-1} dry weight soil [27], whereas the soil samples for the BR were amended with 1 mL of distilled water only. The CO_2-C released by SBR and SIR was quantified by the back titration of 1M NaOH against 0.1M HCl, after $BaCl_2$ carbonate precipitation.

Soil microbial biomass was determined with the fumigation extraction method, specifically microbial biomass C (B_C) according to [28] and microbial biomass N (B_N) according to [29]. The metabolic quotient (qCO_2) values were determined by the ratio between SBR and B_C values, and expressed as mg CO_2 mg B_C^{-1} day^{-1} [30].

Alkaline and acid phosphatase activities were determined with the method of Tabatabai and Bremner [31], the phosphodiesterase activity according to Browman and Tabatabai [32], the β-glucosidase activity was measured according to Tabatabai [33]. The urease activity was assayed by the method of Nannipieri et al. [34], and the protease activity using N-benzoylargininamide as substrate [35]. All enzyme activities were assayed at 37 °C for 1 h, except the protease activity that was assayed for 2 h. Concentrations of 4-nitrophenol (4-NP) produced in the assays of acid and alkaline phosphomonoesterase and phosphodiesterase and β-glucosidase activities were calculated from the absorbance of light at 400 nm wavelength by UV/VIS spectrophotometry using a 4-NP calibration curve. The NH_4^+-N released by urease and protease activities was extracted with 1M KCl and quantified by spectrophotometry at 660 nm after reaction with the Nessler reagent.

2.4. Data Analysis

Data was analyzed by one-way ANOVA followed by the Fisher PLSD post-hoc test using the Statview software (SAS Institute), and reported as means (*n* = 5) ± standard deviation. The difference between mean values with a *p* value <0.05 was considered significant.

3. Results

3.1. Soil Chemical Properties

Main physico-chemical properties of de-sealed and agricultural soils are reported in Table 1. The BD values of the DS2 and DS20 soils were significantly higher than those of the DS34 and AS

(Table 1). The pH values of the de-sealed soils showed progressive significant acidification upon increasing of de-sealing time, whereas the arable soil showed a sub-acidic pH value, with a significantly lower value than that of the de-sealed soils (Table 1). The EC values were significantly higher in the arable as soil compared to the de-sealed soils, and among the latter the DS20 and DS34 soils showed higher values than the DS2 soil (Table 1). The CEC showed significantly higher values for all the de-sealed soils than for the arable soil (Table 1). The TOC and TN contents were higher in the de-sealed soils than in the arable soil, whereas the TEC contents showed higher values in the DS34, followed by DS20 and AS soils with similar values, whereas the DS2 soil showed the lowest TEC value (Table 1). Concerning the NH_4^+-N showed the trend DS2 > DS20 = DS34 > AS, whereas the NO_3^--N content showed the following trend: DS34 > DS20 = AS > DS2 (Table 1).

Table 1. Main physico-chemical properties of the arable soil and de-sealed soils.

Soil	BD (g cm^{-3})	pH	EC (µS cm^{-1})	CEC (cmol kg^{-1})	TOC (g kg^{-1})	TEC (g kg^{-1})	TN (g kg^{-1})	NH$_4^+$-N (g kg^{-1})	NO$_3^-$-N (g kg^{-1})
AS	1.05 ± 0.04 [c]	6.85 ± 0.03 [d]	230 ± 11.9 [a]	22.9 ± 0.92 [a]	12.2 ± 0.31 [c]	703 ± 28.1 [b]	1.01 ± 0.06 [c]	4.73 ± 0.29 [c]	29.7 ± 3.90 [b]
DS2	1.25 ± 0.05 [a]	7.20 ± 0.03 [a]	164 ± 5.63 [c]	18.6 ± 0.95 [b]	17.2 ± 0.24 [b]	302 ± 13.2 [c]	1.22 ± 0.06 [b]	6.57 ± 0.31 [a]	22.3 ± 1.04 [d]
DS20	1.16 ± 0.02 [b]	7.12 ± 0.02 [b]	192 ± 10.1 [b]	19.0 ± 0.68 [b]	17.1 ± 0.29 [b]	745 ± 50.6 [b]	1.30 ± 0.04 [a]	5.84 ± 0.24 [b]	33.0 ± 1.30 [b]
DS34	1.09 ± 0.05 [c]	7.05 ± 0.03 [c]	194 ± 7.44 [b]	19.0 ± 0.80 [b]	17.6 ± 0.41 [b]	1010 ± 52.3 [a]	1.29 ± 0.03 [a]	5.71 ± 0.40 [b]	40.0 ± 3.33 [a]

BD—Bulk density; EC—Electrical conductivity; CEC—Cation exchange capacity; TOC—Total organic carbon; TEC—Total extractable carbohydrates; TN—Total nitrogen. Values are the means of 5 replicates ± the standard deviation. Different superscripts among mean values of each row indicate significant differences ($p < 0.05$).

### 3.2. Soil Respiration, Soil Microbial Biomass, SIR, and Soil Enzyme Activities

The SBR and SIR values were significantly higher in the DS34 and DS20 soils than in the DS2 and AS soils, whereas the DS2 soil showed the lowest SBR values (Table 2). After subtraction of the of the SIR by the SBR values, the glucose-derived C amounted to 47% for the DS34, 36% for the DS20, 34% for the AS and 21% for the DS2 soils. The highest B_C content was found in the DS34 soil followed by DS20 and AS soils which had similar values, whereas the B_C content was found in the DS2 soil (Table 2). The B_N values showed the similar ranking, except that the DS20 soil showed a higher value that of the AS soil (Table 2). The qCO_2 values showed higher values in the DS34 soil followed by the DS20, AS and DS2 soils, whereas the B_C-to-TOC ratio values showed higher values in the DS2, followed by DS34 and DS20 soils with similar values, followed by the AS and soil showing the lowest value (Table 2).

Table 2. Basal respiration of the arable soil and de-sealed soils.

Soil	SBR (mg CO_2-C kg^{-1})	SIR (mg CO_2-C kg^{-1})	B_C (mg C kg^{-1})	B_N (mg N kg^{-1})	qCO_2 (mg CO_2-C mg BC^{-1})	B_C/TOC (%)
AS	48.4 ± 4.21 [c]	722 ± 34.6 [c]	341 ± 31.8 [b]	30.3 ± 3.64 [c]	0.036 ± 0.02 [c]	2.69 ± 0.21 [c]
DS2	7.79 ± 0.21 [d]	429 ± 54.5 [d]	199 ± 10.0 [c]	13.4 ± 1.97 [d]	0.010 ± 0.01 [d]	5.60 ± 0.14 [a]
DS20	78.8 ± 8.12 [b]	809 ± 35.5 [b]	380 ± 24.5 [b]	41.7 ± 1.72 [b]	0.052 ± 0.01 [b]	4.57 ± 0.22 [b]
DS34	174 ± 6.25 [a]	1112 ± 59.9 [a]	580 ± 30.4 [a]	49.7 ± 2.35 [a]	0.075 ± 0.04 [a]	4.26 ± 0.26 [b]

SBR—Soil basal respiration; SIR—Substrate induced respiration; B_C—Biomass C; B_N—Biomass N; qCO_2—Metabolic quotient; TOC—Total organic carbon. Values are the means of 5 replicates ± the standard deviation. Different superscripts among mean values of each row indicate significant differences ($p < 0.05$).

The DS34 and the DS2 soils showed the highest and the lowest values for all the measured enzyme activities, respectively (Figure 1). For the other two analyzed soils, the DS20 showed higher values of the alkaline and acid phosphatase activities than the AS soil, while the AS soil showed higher protease, urease and β-glucosidase activities than the DS20 soil, and the phosphodiesterase and arylsulfatase activities showed similar values in the two soils (Figure 2).
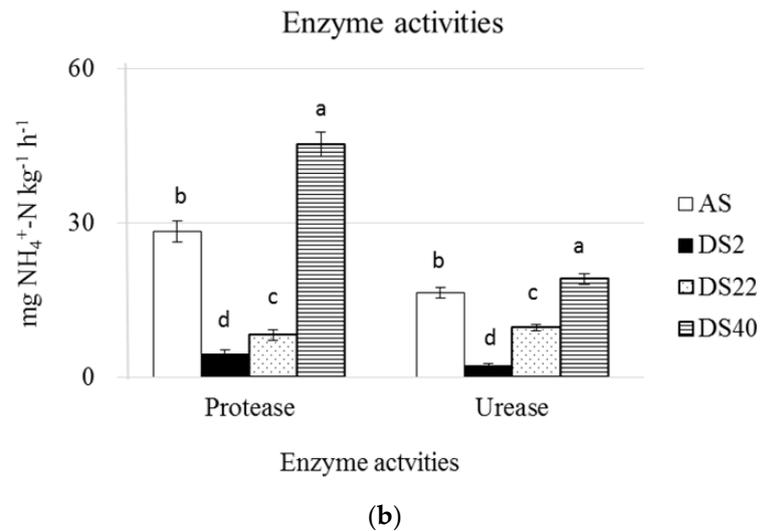

(**a**)

Figure 1. *Cont.*

(**b**)
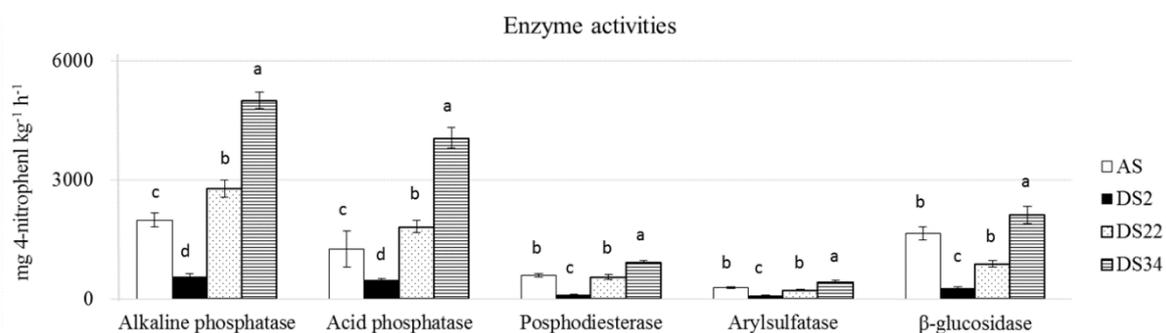
Figure 1. Enzyme activities of the arable soil (**a**) and de-sealed soils (**b**). For each enzyme activity, different superscripts indicate significant differences ($p < 0.05$) between the mean values ($n = 5$).
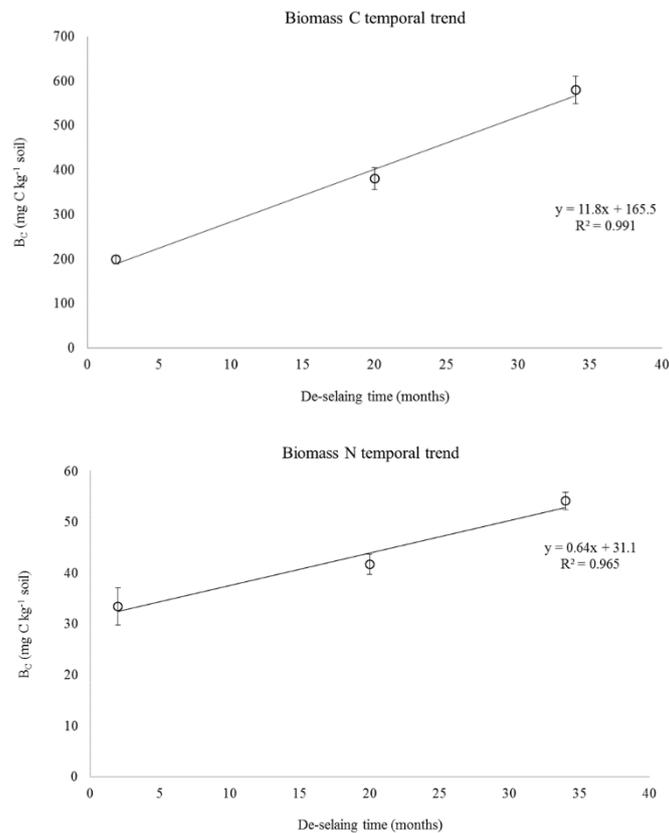

Figure 2. Trend of the microbial biomass C in soil de-sealed for 2, 20 and 34 months. The error bar represents the standard deviation of the mean values ($n = 5$).

4. Discussion

De-sealing for increasing times led to a significant decrease the soil BD, likely due to the surface colonization by volunteer plants, indicating a reduced soil compaction and an improved soil structure, factors of prime importance for the rehabilitation of soil ecological functions [36]. Improved structure of the soil and plant colonization were likely joint factors influencing the C and N pools, particularly the increased TEC and NO_3^--N content of the de-sealed soils upon time (Table 1). With the rhizodepositions

in fact, plants release sugars, amino acids, low molecular weight organic acids and phenolics, along with sloughed cells and mucigels, which are rapidly mineralized by soil microorganisms, thus sustaining a larger and more active microbial community in the plant rhizosphere [37]. The TEC results support the previous finding that forest soils contain significantly higher carbohydrate content than arable soil [38]. Decomposition of rhizodepositions and TEC led the significant acidification of the DS34 and DS20 soils as compared to the DS2 soil, owing to the production of H^+ and nitrification activity, as previously reported for a plant colonization chronosequence [39]. Increase in TEC, CEC and NO_3^--N of DS20 and DS34 soils as compared to the DS2 soil was in line with data reported for a rhizosphere soil during a vegetation chronosequence [40].

Enrichment of the de-sealed soils with labile C resulted in increased basal respiration, total microbial biomass and fungal biomass sustained by fresh inputs of plant debris into soil [41], and the increases of BR, MBC, and MBN were in agreement with previous reports [42]. The increase of microbial activity was also reflected in the higher SIR response and higher soil enzyme activities with increasing of de-sealing time, as well as the higher qCO_2 values observed in the DS34 and DS20 as compared to the DS2 soil (Table 1, Figure 1). The B_C/TOC ratio values showed typical previously reported values for the AS [43], whereas the significantly higher values of the DS soils indicated an accumulation in the sealed soils due to reduced SOM decomposition. In this study, the B_C/TOC ratio values were 3.2% for the DS34, 2.8% for the AS, 2.2% for the DS20 and 0.9% for the DS2 soils. Average B_C/TOC ratio value of arable and forest soils are in the range 1–5% [44], and it varies only in soils after fresh organic inputs [45]. Soil B_C and B_N values of urban soils are profoundly influenced by soil physical properties such as water infiltration soil temperature, concentration of nutrients and pollutants, and in urban soils human disturbance leads to altered values and wide fluctuations as compared to agricultural soil [46,47].

The metabolic quotient (qCO_2) values of the DS soils significantly increased upon increasing of de-sealing time (Table 2). Increased qCO_2 values have been often as indicators of a stress response of soil microorganisms leading to a lower C use efficiency caused by microbial selection or energy diversion in the microbial community from cell proliferation to repair of cell damages [48]. However, while this interpretation can be valid for long-term impacted soils under steady state, it is unlikely to apply to the present study. In fact, the studied de-sealed soils could be considered still under evolution upon colonization by volunteer plants, in which soil microorganisms could still increase in biomass and diversity upon soil enrichment of plant-borne labile C. This was confirmed by the linear increase of B_C and B_N with time (Figure 2). In this sense, the DS34 and DS20 soils could be considered as energy rich ecological habitats under microbial colonization sustained by plant rhizodepositions. Kuzyakov and Domanski [49] reported that up to 20% of C assimilated by young plants of wheat, barley, or grasses could be retrieved in the SOM pool available to soil microorganisms, and even higher percentages have been reported using ^{14}C-labeling techniques of rhizodepositions [50]. Therefore, the significantly higher qCO_2 values found in the DS soils upon increasing of the de-sealing time reflected proliferation of soil microorganisms [51]. Overall, this microbial anabolic mechanism is at the basis of the microbial C pump mechanism [52] that can lead to C stabilization and sequestration in de-sealed revegetated soils.

Among the measured soil enzyme activities, higher protease and urease activities catalyzing organic N mineralization could explain the increased NO_3^--N content of the DS34 and DS20 soils, as the release of ammonia-N in well aerated soils generally fuels the nitrification [53]. Increase of β-glucosidase activity upon soil revegetation confirms previous reports, showing that this enzyme activity is generally higher high in grassland soils [54,55], and is an indicator of soil quality [56], and the general increase of soil enzyme activities in involved in C, N, P and S cycles in the DS34 and DS20 soils to higher or equivalent levels to those of an agricultural soil could be ascribed to the increase of labile C (TEC) content and higher microbial biomass and metabolic activity [57].

5. Conclusions

Soil de-sealing improved their physical and chemical fertility in a relatively short time, and increased the soil microbial biomass and biochemical activity to levels comparable or higher than those of an agricultural soil of the same area. Such an improvement occurred with no intervention and was favored by the colonization by volunteer plants that allocated labile C into soil. These results can contribute to the recent growing interest in assessing the quality of urban soils and support the hypothesis that soil de-sealing, even followed by plant colonization, improve soil ecosystem services and quality of the urban environment while storing C in soil.

Conflicts of Interest: The authors declare no conflict of interest.

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