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# Carbon and phosphorus biogeochemical cycles in native forest and horticultural areas in the Metropolitan Region of Curitiba, Brazil

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**Abstract**
This study was carried out to understand the dynamics of carbon and phosphorus biogeochemical cycles in native forest and horticultural areas. Soil samples were collected from native forest and horticultural areas, in four municipalities in the Metropolitan Region of Curitiba, Brazil, and evaluated for: carbon, nitrogen and phosphorus of soil microbial biomass (MBC, MBN and MBP, respectively), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), inorganic phosphorus (iP), organic phosphorus (oP) and available phosphorus (aP. Soil suspensions diluted at $10^{-4}$ were spread on plates and phosphate solubilizing bacteria (PSB) were counted. The analyses showed that horticultural areas soils accumulated 43% more TP whereas they lost 23% of TOC and 19% of TN comparing to native areas. 69% of TP in the native areas was organic (oP) whereas 59% of TP in the horticultural areas was inorganic (iP). Horticultural areas had lower numbers of colony forming unities of PSB than native areas. PSB was positively correlated with the ratio of MBC to TOC (qMic), which in turn, was negatively correlated with TOC and TN. Changes in the soil P fractions suggested a shift in the soil community bacterial structure and in the values of soil microbial biomass of the two different soil ecosystems. The excessive P addition may stimulate soil microbial attack to soil organic matter reserves, which may have consequences for maintenance of soil quality and agriculture sustainability.
**Keywords:** P solubilizing bacteria; soil intensive use; soil organic matter; vegetables production.

## Ciclos biogeoquímicos de carbono e fósforo em áreas de floresta nativa e horticultura na Região Metropolitana de Curitiba, Brasil

**Resumo**
Este estudo foi realizado com o objetivo de compreender a dinâmica dos ciclos biogeoquímicos do carbono e do fósforo em áreas de floresta nativa e horticultura. Amostras de solo foram coletadas em áreas de floresta nativa e horticultura, em quatro municípios da Região Metropolitana de Curitiba, Brasil, e avaliadas quanto a: carbono, nitrogênio e fósforo da biomassa microbiana (MBC, MBN e MBP, respectivamente), carbono orgânico total (TOC), nitrogênio total (TN), fósforo total (TP), fósforo inorgânico (iP), fósforo orgânico (oP) e fósforo disponível (aP). As suspensões de solo foram diluídas até $10^{-4}$ e espalhadas em placas de Petri, e, as bactérias solubilizadoras de fosfato (PSB) foram contadas. As análises mostraram que os solos das áreas de horticultura acumularam 43% a mais de TP enquanto perderam 23% de TOC e 19% de TN, em comparação com as áreas nativas. 69% do TP nas áreas nativas era orgânico (oP) enquanto 59% do TP nas áreas de horticultura era inorgânico (iP). As áreas de horticultura apresentaram menor número de unidades formadoras de colônias de PSB do que as áreas nativas. PSB foi positivamente correlacionado com a razão de MBC para TOC (qMic), que por sua vez, foi negativamente correlacionado com TOC e TN. Mudanças nas frações de P do solo podem ter levado a uma mudança na estrutura bacteriana da comunidade do solo e nos valores de biomassa microbiana do solo. A adição excessiva de P pode estimular o ataque microbiano do solo às reservas de matéria orgânica do solo, o que pode ter consequências para a manutenção da qualidade do solo e sustentabilidade da agricultura.
**Palavras-chave:** bactérias solubilizadoras de P; uso intensivo do solo; matéria orgânica do solo; produção de hortaliças.

**Introduction**

Phosphate is one of the major nutrients required by living organisms, but its availability is limited in tropical and subtropical soils around the world. The availability of P in soil solution results from the process of solubilization from minerals and mineralization of soil organic matter in the P biogeochemical cycle (EHLERS *et al*., 2010; HARTMAN; RICHARDSON, 2013; HEUCK *et al*., 2015; SPOHN; CHODAK, 2015; LI *et al*., 2015). The concentration of available P in the soil solution is usually low because the ion $PO_4^-$ is highly attracted by mineral surfaces, and because soil microrganisms and plants compete for the same pool in the absorption process (EHLERS *et al*., 2010; HARTMAN; RICHARDSON, 2013; HEUCK *et al*., 2015; SPOHN; CHODAK, 2015; LI *et al*., 2015).

Plants may deal with P limitation by releasing organic acids in the rhizosphere to solubilize inorganic P (HOFFLAND *et al*., 1989), by increasing the production and release of phosphatase that breakdown organic P (YUN; KAEPPLER, 2001), by increasing root extension and architecture (NIU *et al*., 2013), by stablishing partnership with mycorrhizal fungi (SMITH *et al*., 2011) and other plant growth promoting microrganisms (KAFLE *et al*., 2019). Despite the strong binding of P in solid soil particles, soil microorganisms may increase P availability in the soil because they release organic acids and protons that decrease soil pH and weaken chemical bonds between $H_2PO_4^-$ and -FeOH and -AlOH (RICHARDSON, 2001; RICHARDSON; SIMPSON, 2011; GLICK, 2012; SHARMA *et al*., 2013; ZHU *et al*., 2018; YAO *et al*., 2018). The P absorbed by roots and microorganisms is incorporated into organic P (oP). Organic P represents 5-80 % of total P in the soil (TP) (BROOKES *et al*., 1982) and can be mineralized and recycled through enzymatic (phosphatase) activity (RICHARDSON; SIMPSON, 2011; SHARMA *et al*., 2013). On one hand, soil microbial growth is coupled with mineralization of P and it may be one of the major process that makes P available for root uptake (GERKE, 2015). On the other hand, microbial growth in soil increases the immobilization of P, by increasing microbial biomass P (MBP), even though immobilization is a transient inconvenient because P may become available again after the microorganisms death (BROOKES *et al*., 1982; BALOTA *et al*., 2003).

The soil microbial biomass, considered as the living fraction of soil organic matter, composed of bacteria, archaea, fungi, algae, protozoa and other organisms smaller than 5 x $10^3$ $\mu m^3$, is also limited by other nutrients (e.g. carbon, nitrogen, sulfur) because the nutrients cycling processes are coupled with the process of soil organic matter decomposition. The soil microbial biomass represents 2 to 5 % of total organic carbon (TOC), 1 to 5% of total nitrogen (TN) and 5 to 80 % of total P (TP) depending on the soil conditions (VANCE *et al*., 1987; BROOKES *et al*., 1982; BROOKES *et al*., 1985). It is expected that the maximum growth of soil microbial biomass is determined by the nutrient that is less available in the soil solution (HARTMAN; RICHARDSON, 2013; HEUCK *et al*., 2015; SPOHN; CHODAK, 2015; LI *et al*., 2015; YAO *et al*., 2018). Therefore, although it is emphasized that TOC and TN are limiting factors for the soil microbiota growth (SPOHN; CHODAK, 2015), the total P (TP) is also crucial (LI *et al*., 2015; YAO *et al*., 2018).

The Metropolitan Region of Curitiba is composed by 29 municipalities (over 3.5 million inhabitants) and occupies 16,581 $km^2$, being 44 % with small-sized farms (< 3.5 ha) managed by familiar farming (IPARDES, 2004) that, in 2016, produced 1,196,593 tons of vegetables (SEAB, 2016). The horticultural production systems have operated within urban, peri-urban, and legal units of biodiversity conservation, with intense soil preparation that increases the risks of erosion and pollution by eutrophization (RAMOS *et al*., 2014). Since vegetables are fast-growing crops and have a high demand for nutrients, farmers commonly apply high doses of fertilizers and external organic waste (e.g. poultry litter) whose excess is accumulated in the soil (RAMOS *et al*,. 2014). Therefore, while P limitation is an issue over the world, in metropolitan regions like the Metropolitan Region of Curitiba, excessive P addition may become a potential environmental issue. Intensive agriculture may result in soil erosion (RAMOS *et al*., 2014), and, an hypothesis is that excessive P addition may stimulate soil microbial attack to soil organic matter reserves, which, may have consequences for maintenance of soil quality and agriculture sustainability.

Considering that the nutrients biogeochemical cycle are interconnected within several processes and that the soil microbial biomass may influence the P biogeochemical cycle but may be affected by P availability as well, this study was conducted to verify whether changes in soil P fractions from native forest areas to

horticultural areas may affect the nutrient fractions of soil microbial biomass, and whether this changes are correlated to soil organic matter contents.

## Material and Methods

### Experimental sites

Soil samples were collected in twelve sites in the municipalities of Colombo, Rio Branco do Sul, Itaperuçu and Campo Magro in the Metropolitan Region of Curitiba, South Brazil (Tables 1 and 2), between September and November 2016. The region is characterized by altitudes varying between 730 and 1026 m, subtropical humid climate - Cfb according to Köppen-Geiger climate classification, mean annual temperatures lower than 21 °C, thermal amplitude between 9 °C and 23 °C, total annual precipitation between 1,300 and 1,800 mm and well distributed rains throughout the year. The bedrock in the area is mostly clayey and the soils are poorly developed (known as Cambissolos in the Brazilian Soil Classification System (SANTOS *et al*., 2018; and as Inceptisols in the USDA Soil Taxonomy). All sites were located within small farms that produce and market vegetables throughout the year. Soil samples were collected from side-by-side horticultural and fragments of native secondary forest areas having soils with similar origin, at the same topographic level and the same position in relation to the sunshine.

**Table 1.** Sample sites in the Metropolitan Region of Curitiba*.

| Site | Altitude (m) | Coordinates | Fertilizer (mineral N-P-K plus…) | Relief |
|---|---|---|---|---|
| **1** | 703 | 25°05'10.19"S; 49°34'72"W | stove ash | Wavy |
| **2** | 641 | 25° 4'34.46"S; 49°34'34.93"W | no one | Wavy |
| **3** | 1001 | 25°15'0.11"S; 49°12'55.64"W | no one | Wavy |
| **4** | 880 | 25°17'49.23"S; 49°26'8.10"W | poutry litter | Wavy |
| **5** | 782 | 25°16'26.65"S; 49°27'41.40"W | poutry litter | Wavy |
| **6** | 787 | 25°16'25.86"S; 49°27'40.43"W | poutry litter | Soft Wavy |
| **7** | 980 | 25°13'23.78"S; 49°14'29.09"W | poutry litter | Soft Wavy |
| **8** | 1026 | 25°14'44.94"S; 49°13'36.62"W | poutry litter | Wavy |
| **9** | 972 | 25°13'38.43"S; 49°14'19.19"W | poutry litter | Wavy |
| **10** | 1020 | 5°14'43.65"S; 49°13'30.96"W | poutry litter + soluble fertilizer | Wavy |
| **11** | 1019 | 25°14'6.04"S; 49°14'24.38"W | poutry litter + soluble fertilizer | Wavy |
| **12** | 1007 | 25°14'6.32"S; 49°14'41.80"W | poutry litter + soluble fertilizer | Soft Wavy |

* Soils were classified as Cambisol by the Brazilian System of Soil Taxonomy (Santos et al. 2018). Forest native vegetation were classified as Mixed Ombrophilous Forest under secondary formation. Traditionally, farmers allow bovine cattle to graze lower vegetation inside the forest fragments. The horticultural systems included rotation of short-cycle vegetables (cabagge, lettuce, carrot, radish, tomato, etc.) in rotation during the year. Farmers apply N-P-K fertilizers and occasionally other source of nutrients, but not with a fixed calendar. Therefore, readers are asked to use Table 2 to know the levels of soil fertility during the study.

**Table 2.** Soil chemical attributes and clay contents in horticultural and native areas of the Metropolitan Region of Curitiba.

| Site | pH | | $Al^{3+}$ | | H + Al | | $Ca^{2+}$ | | $Mg^{2+}$ | | $K^{+}$ | | Clay | | Sand | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NAT | HORT | NAT | HORT | NAT | HORT | NAT | HORT | NAT | HORT | NAT | HORT | NAT | HORT | NAT | HORT |
| | ----- $CaCl_2$ - | | | | | | | | $cmol_c\ dm^{-3}$ | | | | | | $g\ kg^{-1}$ | |
| | -- | | | | | | | | | | | | ----- | | | |
| **1** | 3.66 | 5.51 | 3.54 | 0.04 | 16.13 | 3.90 | 0.73 | 4.23 | 0.69 | 1.90 | 0.13 | 0.27 | 533 | 400 | 263 | 421 |
| **2** | 5.73 | 5.41 | 0.03 | 0.04 | 4.20 | 4.77 | 7.77 | 8.46 | 2.28 | 2.90 | 0.62 | 0.32 | 342 | 371 | 417 | 413 |
| **3** | 5.14 | 6.31 | 1.28 | 0.01 | 6.90 | 3.53 | 5.60 | 9.40 | 4.30 | 4.03 | 0.20 | 0.98 | 467 | 417 | 283 | 292 |
| **4** | 4.53 | 5.81 | 0.17 | 0.00 | 8.20 | 3.73 | 2.83 | 5.18 | 1.79 | 2.61 | 0.10 | 0.47 | 313 | 238 | 225 | 121 |
| **5** | 5.36 | 5.83 | 0.02 | 0.14 | 5.20 | 4.17 | 5.53 | 5.92 | 3.47 | 2.97 | 0.29 | 1.40 | 375 | 404 | 354 | 308 |
| **6** | 5.32 | 6.34 | 0.03 | 0.00 | 5.00 | 3.13 | 3.59 | 5.58 | 3.22 | 3.25 | 0.19 | 1.14 | 275 | 329 | 138 | 129 |
| **7** | 4.35 | 5.20 | 0.54 | 0.06 | 9.67 | 5.83 | 3.27 | 6.51 | 1.30 | 2.26 | 0.08 | 0.13 | 454 | 454 | 225 | 204 |
| **8** | 4.40 | 5.43 | 0.62 | 0.29 | 9.57 | 4.73 | 2.39 | 3.43 | 1.49 | 1.13 | 0.17 | 0.51 | 475 | 413 | 238 | 171 |
| **9** | 4.96 | 6.19 | 0.03 | 0.00 | 5.53 | 3.10 | 4.02 | 4.35 | 2.02 | 2.31 | 0.28 | 0.89 | 283 | 383 | 275 | 183 |
| **10** | 5.17 | 6.04 | 0.29 | 0.02 | 8.23 | 3.03 | 5.52 | 4.11 | 3.58 | 2.65 | 1.04 | 1.40 | 396 | 354 | 308 | 542 |
| **11** | 5.95 | 6.01 | 0.00 | 0.02 | 4.13 | 4.13 | 8.22 | 5.76 | 3.73 | 3.47 | 0.50 | 0.49 | 458 | 542 | 200 | 196 |
| **12** | 6.05 | 6.22 | 0.00 | 0.00 | 4.00 | 3.63 | 6.80 | 7.12 | 3.77 | 3.48 | 0.23 | 1.66 | 383 | 367 | 142 | 133 |
| **Average** | **5.05** | **5.86** | **0.55** | **0.05** | **7.23** | **3.98** | **4.69** | **5.84** | **2.64** | **2.75** | **0.32** | **0.80** | **349** | **352** | **397** | **389** |

* Mean of three samples.

**Soil samples**

Three composite soil samples were collected at 0-20 cm depth and packed in polyethylene bags. The samples were divided into two subsamples. The first one was cleaned from fragments of plants and remains of soil fauna, sieved in a 2 mm-mesh and stored at 4 °C for microbiological analyses done before a period of 50 days under storage. The second subsample was oven dried at 40 °C until constant weight and submitted to chemical and physical analyses.

**Physical and chemical analyses**

Soil particle size was determined with the Bouyoucos densimeter method (GEE; BAUDER, 1986). Soils chemical analyses were performed according to the methods of Soil Survey Laboratory Staff (USDA-NRCS, 1996), by extracting exchangeable $Ca^{+2}$, $Mg^{+2}$ and $Al^{+3}$ with 1 mol $L^{-1}$ KCl, exchangeable $K^{+}$ with Mehlich-I solution, and measuring soil pH in 0.01 mol $L^{-1}$ $CaCl_2$. Total nitrogen (TN) and total organic carbon (TOC) were determined by dry combustion in elemental analyzer (CHNOS), model Vario El III - elementar®, Germany.

**Carbon and Nitrogen of the Microbial Biomass (MBC and MBN)**

Carbon and nitrogen of the microbial biomass were measured using the fumigation-extraction method modified by Vance *et al*. (1987) and Brookes *et al*. (1985). Analyses were performed using two soil subsamples. The first subsamples (20 g moist soil) were placed in vials into a hermetically sealed A36 carbon steel box, containing other three 50 mL vials containing water (non-fumigated soil) and another three 50 mL vials containing ethanol–free chloroform for fumigation, and, then submitted to vacuum (600 mmHg) for 24 hours. After incubation, chloroform was removed from soil by intermittent vacuum, for 3 minutes, 5 times. The second subsample (20 g moist soil) was let to rest in the dark for extraction. The soil was added to 50 mL of potassium sulfate solution (0.5 M $K_2SO_4$), shaken for 60 minutes in an orbital shaker, centrifuged at 2,500 rpm for 10 minutes and finally filtered. The supernatant corresponding to intracellular material was stored at -20 °C until analysis, not exceeding 30 days.

The extract C was determined according to Bartlett and Ross (1988) using a spectrophotometer (Shimadzu UVmini-1240 Inc, Kyoto, Japan) with wavelength of 495 nm. The MBC was calculated by the difference between fumigated and non-fumigated samples (mg C $kg^{-1}$ soil), using a $k_C$ correction factor of 0.41 (ANDERSON; DOMSCH, 1978).

Nitrogen was determined after digestion with concentrated $H_2SO_4$ at 350 °C (Bremner, 1965). Ammoniacal N (N-$NH_4$) was obtained by colorimetry with indophenol blue, according to Feije and Anger (1972). Absorbances were measured in spectrophotometer (Shimadzu UVmini-1240 Inc, Kyoto, Japan) with a wavelength of 630 nm. Microbial biomass N (MBN) was

calculated like MBC, but using a $k_N$ correction factor of 0.54 (BROOKES *et al*., 1985).

**P in the Microbial Biomass (MBP)**

The MBP was obtained by fumigation-extraction method (BROOKES *et al*., 1982), modified by Vance *et al*. (1987), using two samples of 10 g moist soil. Fumigation was done as described for MBC, but extraction of P was performed with sodium bicarbonate (0.5 M $NaHCO_3$). After extraction, 20 mL aliquots of the extracts were transferred to digestion tubes, amended with 2 mL perchloric acid and 1 mL of $MgC_2$ and then boiled until they were colorless. The digested extract P was obtained by blue molybdate method (MURPHY; RILEY, 1962), using a wavelength of 660 nm. MBP was calculated like MBC, but using a $k_P$ correction factor of 0.40 (ANDERSON; DOMSCH, 1978).

**P solubilizing bacteria (PSB)**

Fresh soil samples of each experimental unit were submitted to serial dilution with NaCl 0.85 % (up to $10^{-4}$). The diluted samples were then spread on three Petri dishes with dextrose-yeast medium containing unsoluble phosphate (KATZNELSON; BOSE, 1959) to count the number of colony-forming unit (CFU) of total bacteria and P solubilizing bacteria (PSB). Petri dishes were incubated by inversion at 25 °C for 12 days. Colonies forming a transparent halo around their central axis were counted for PSB.

**Soil Total P, inorganic P, organic P and available P**

Soil phosphate (P) fractions were determined in different extracts obtained from the same soil sample. Total P (TP) and inorganic P (iP) were obtained from 2 g moist soil, corrected to dry weight, according to Olsen and Sommers (1982). TP extracts were obtained from 2 g of dry soil samples (45 °C in oven for 24 h) placed in porcelain crucibles, heated in a muffle at 550 °C for 1 hour, and resuspended in 50 mL $H_2SO_4$ 0.5 mol $L^{-1}$. The iP extracts were obtained from non-heated soil samples placed in glass jars, suspended in 50 mL $H_2SO_4$ 0.5 mol $L^{-1}$ and shaked in horizontal stirrer at 220 rpm for 16 hours. In both determinations (TP and iP), soil extracts were filtered through quantitative paper filter (nominal retention of 2 micra). P content in each extract was determined in an inductively coupled plasma optical emission spectrometer (ICP-OES VARIAN 720-ES). Organic P (oP) was estimated after ignition (Olsen and Sommers, 1982) from the difference between TP and iP. Available P (aP) was extracted with Mehlich-I solution ($H_2SO_4$ 0.0125 mol $L^{-1}$ + HCl 0.05 mol $L^{-1}$).

**Experimental design and statistical analysis**

Records of MBC, MBN, MBP, number of PSB colony-forming units and soil chemical attributes were submitted to Shapiro Wilk test to confirm ANOVA assumptions. ANOVA was performed in a completely randomized experimental design, in which, the two treatments (native versus horticultural areas) were represented by 12 (sites) times 3 (replicates) independent samples. Pearson correlations were generated within each treatment, with 95% confidence interval. Statistical analyses were performed using the R environment (RSTUDIO TEAM, 2016) and Microsoft Excel® 2010.

**Results**

The conversion of native areas into horticultural fields modified the soil chemical and biological attributes and altered biogeochemical cycling processes. Analyses of soil total and fractional contents C, N and P evidenced that horticultural systems altered the fractions and the ratios of these nutrients in relation to native forest areas (Table 3). The correlations between variables (Table 4) indicate that the modified soil C, N and P fractions in horticultural areas probably caused alterations in the microorganisms functioning on the P biogeochemical cycle.

**Carbon and Nitrogen soil fractions**

Soils in the horticultural areas lost an average of 38% of the organic carbon (TOC) compared to native areas (Table 3), with few differences in trends among the 12 sampling sites (data not shown). The low TOC values followed the low values of total nitrogen (TN) so that, horticultural areas contained 31% less TN than native areas. The soils of the horticultural areas had a lower C/N ratio (8%) than the native areas (Table 3), in spite of positive correlations of TOC with TN increases. The MBP content was not related to the TOC content, TN and C / N ratio (Table 4).

**Table 3.** Soil biological attributes, soil P fractions and soil chemistry ratios in native and horticultural areas of the Metropolitan Region of Curitiba.

| Biological attributes | Unities | NAT | CV(%) NAT | HORT | CV(%) HORT | Index of Change (%) |
|---|---|---|---|---|---|---|
| TOC (Total Organic Carbon) | g C $kg^{-1}$ soil | 45.92 a | 27.5 | 28.57 b | 42.8 | -23.3 |
| TN (Total Nitrogen) | g N $kg^{-1}$ soil | 3.92 a | 106.1 | 2.67 b | 65.3 | -19.0 |
| C/N (Soil C/N Ratio) | % | 11.43 a | 79.4 | 10.43 a | 78.2 | -4.6 |
| MBC (Microbial Biomass-C) | mg C $kg^{-1}$ soil | 325.18 a | 29.6 | 222.81b | 46.4 | -18.7 |
| MBN (Microbial Biomass-N) | mg N $kg^{-1}$ soil | 96.22 a | 69.9 | 62.54 b | 54.5 | -21.2 |
| MBP (Microbial Biomass-P) | mg P $kg^{-1}$ soil | 38.88 a | 6.4 | 61.11 a | 13.5 | 22.2 |
| qMic (Microbial quotient) | % | 0.76 a | 32.4 | 0.85 a | 20.0 | 5.6 |
| TB (Total bacteria) | CFU 100 $g^{-1}$ soil | 11141.67 a | 158.0 | 11807.41 a | 178.0 | 2.9 |
| PSB (P Solubilizing Bacteria) | CFU 100 $g^{-1}$ soil | 131.94 a | 19.8 | 73.10 b | 46.2 | -28.7 |
| **Fractions of soil phosphate** | | | | | | |
| TP (total phosphate) | mg P $kg^{-1}$ soil | 388.66 b | 45.7 | 979.20 a | 46.0 | 43.2 |
| iP (inorganic phosphate) | mg P $kg^{-1}$ soil | 135.80 b | 45.7 | 709.63 a | 46.0 | 67.9 |
| oP (organic phosphate) | mg P $kg^{-1}$ soil | 252.85 a | 70.6 | 269.57 a | 62.0 | 3.2 |
| aP (available phosphate) | mg P $kg^{-1}$ soil | 15.29 b | 32.4 | 100.77 a | 20.0 | 73.7 |
| **Soil chemistry ratios** | | | | | | |
| iP/TP | unitless | 0.349 b | 45.7 | 0.725 a | 46.0 | 35.0 |
| oP/TP | unitless | 0.651 a | 30.9 | 0.275 b | 24.5 | -40.6 |
| MBP/TP | unitless | 0.100 a | 51.0 | 0.064 a | 64.2 | -22.0 |

* Means of 36 samples. Index of change={(Horticultural-Native)/(Horticultural + Native)}*100, where "Horticultural" were the means of measurements in horticultural soil samples and "Native" were the means in native forest soil samples. Diferent letters indicate statistical differences at p<0.05 by the *t*-test. n.e. = not estimated. CV(%)= coefficient of variation. HORT= horticultural; NAT=native

**Table 4.** Pearson correlation between soil microbiological attributes and P fractions or total C, total N or C/N ratios in native and horticultural areas of the Metropolitan Region of Curitiba.

| | Native | | | | | | Horticultural | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **PSB** | **TP** | **aP** | **TOC** | **TN** | **C/N** | **PSB** | **TP** | **aP** | **TOC** | **TN** | **C/N** |
| **MBC** | ns | ns | 0.691 | 0.759 | 0.758 | ns | Ns | ns | ns | ns | ns | ns |
| **MBN** | ns | ns | 0.834 | 0.770 | 0.861 | -0.621 | Ns | ns | ns | 0.614 | 0.611 | ns |
| **MBP** | ns | ns | ns | ns | ns | ns | 0.647 | ns | ns | ns | ns | ns |
| **qMic** | 0.638 | ns | 0.831 | -0.702 | -0.658 | ns | 0.563 | ns | ns | -0.588 | -0.562 | ns |
| **TB** | 0.610 | 0.592 | ns | ns | ns | ns | Ns | ns | ns | ns | ns | ns |
| **PSB** | --- | ns | ns | ns | ns | ns | --- | ns | ns | ns | ns | ns |

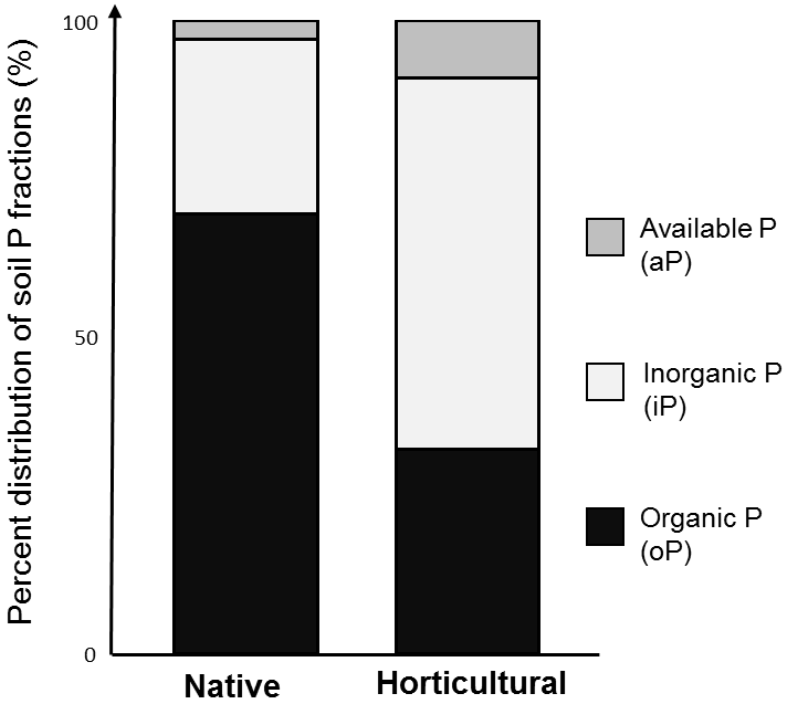
* Values of coefficient of Pearson with significance p≤0.05 by the Student test. ns= not significant at p≤0.05.

**Phosphorus soil fractions**

Soil total P (TP) content considers the sum of inorganic P (iP), organic P (oP), which are the not available fractions, and, available P (aP). The analyses in soil samples from Metropolitan Region of Curitiba showed that soil TP fractions were generally larger in horticultural systems than in native areas (Table 3).

It is interesting to note that in horticultural areas, significant increases in TP were accompanied by significant increases in iP (Table 3; Fig. 1) whereas the highest fraction of TP in native areas was related to oP. In the horticultural areas, iP represented 59% of TP, while oP represented only 32% (Fig. 1). However, in the native areas, iP represented 28% of TP and oP represented 69%.

**Figure 1.** Percent distribution of soil P fractions in native and horticultural systems of the Metropolitan Region of Curitiba. The patterns were obtained by compiling data from 12 independent sites and 36 soil samples.




**P solubilizing bacteria (PSB) and significant correlations**

The number of total bacteria (TB) did not differ significantly between native and horticultural areas (average of 11,500 CFU 100 $g^{-1}$ soil; Table 3).

In native areas, TOC and TN were highly correlated with C/N ratio, MBC and MBN. The correlation between TOC and TN was r = 0.965 (p = 3.6 x $10^{-7}$); the correlation between TOC and MBC was r = 0.759 (p = 0.0042) and MBN was r = 0.770 (p = 0.0034); the correlation between TN and MBC was r = 0.758 (p = 0.0043) and MBN was r = 0.861 (p = 0.0003). In native areas, TOC (r = 0.599, p = 0.0397), MBC (r = 0.691, p = 0.0128) and MBN (r = 0.834, p = 0.0007) correlated with aP. Additionally, MBC (r = 0.692, p = 0.0126) and MBN (r = 0.667, p = 0.0179) correlated with total bacteria (TB). TB showed a positive correlation with TP (r = 0.592; p = 0.0427) and iP (r = 0.700; p = 0.0549) and with PSB (r = 0.610; p = 0.0351).

An interesting point is that PSB correlated with qMic (r = 0.638; p = 0.025) and with iP (r = 0.566; p = 0.054; Table 4). Furthermore, qMic was negatively correlated with TOC and TN in both native and horticultural areas, suggesting that soil P fractions play a role in regulating PSB populations, while these PSB regulate the stocks and consumption of TOC and TN of soils.

**Discussion**

Soil chemical and mineralogical characteristics influence the availability of P in soil solution, but most of the available P in soil solution is the result of biogeochemical cycles of C and N, through the mineralization and immobilization processes by soil microbial biomass (HARTMAN; RICHARDSON, 2013; HEUCK *et al*., 2015; SPOHN; CHODAK, 2015). Soil microbial biomass is a very sensitive soil attribute to soil changes, being affected by climate, land use, roots density and diversity, soil physical and chemical attributes and, by the availability of organic substrate for growth (KASCHUK *et al*., 2010; KASCHUK *et al*., 2011; SARKER *et al*., 2018; VEZZANI *et al*., 2018). If organisms with heterotrophic metabolism grew under a homeostatic paradigm, the increases in the C substrate, i.e. the soil TOC, would result in increases in soil MBC with proportional increases in MBN and MBP (EHLERS *et al*., 2010; HARTMAN; RICHARDSON, 2013; HEUCK *et al*., 2015; SPOHN; CHODAK, 2015; LI *et al*., 2015; YAO *et al*., 2018). However, under varying soil nutrient supplies, soil microbial communities may adjust

their metabolism and composition (EHLERS *et al*., 2010; HARTMAN; RICHARDSON, 2013; HEUCK *et al*., 2015; SPOHN; CHODAK, 2015; UJ, 2015; YAO *et al*., 2018). In this study, the conversion of native into horticultural area decreased soil MBC (microbial biomass-C) by 18% while it increased MBP (microbial biomass-P) by 22% (Table 3). If the homeostatitc paradigm worked in this situation, the conversion of native areas into horticultural areas would stimulate or depress the nutrients at proportional ratios, increases in MBC and MBN would be associated with increases in MBP. However, it seems that the biogeochemical cycles are interconnected and supply of P fertilizers to native soils may accelerate the degradation of soil organic matter reserves.

Our study revealed two different systems in terms of soil P fractions. Horticultural areas received large amounts of P via fertilization, which increased soil TP in relation to native areas. Other studies showed that P availability is a determining factor of soil microbial taxonomy and its structure (HARTMAN; RICHARDSON, 2013; MARANGUIT *et al*., 2017). In this study, increases in soil P fractions of the horticultural areas was not associated with increased MBP (Table 3), but it led to decreases in the number of CFU (colony-forming units ) of PSB (P-solubilizing bacteria) (Table 3). The P solubilization mechanisms by PSB may be exsudation of organic acids that decreases the soil pH and dissociates ferrols and aluminols, and/or exudation of phosphatases that breaks down the organic P (SHARMA *et al*., 2013; ZHU *et al*., 2018). The mechanisms of P solubilization may have contributed to changes in the microbial quotient (ratio of MBC to TOC) in both native and horticultural areas (correlation qMic versus PSB; Table 4).

Several studies have shown that agricultural practices may impair the maintenance of soil organic matter due to temperature and humidity oscillations, factors that influence soil microbial activity (KASCHUK *et al*., 2010; RAMOS *et al*., 2014). Data in this study suggested that soil practices and probably addition of P may have increased the rates of decomposition of soil organic matter, decreasing the contents of soil TOC, TN and C/N contents (Table 3). Since higher values of C and N in the microbial biomass and higher C/N ratio are associated with stability of soil organic matter (SARKER *et al*., 2018; VEZZANI *et al*., 2018), the data herein suggested that horticultural areas are also loosening the stability of soil organic matter. In addition, the analyses revealed negative correlations of qMic (ratio of MBC to TOC) with TOC or TN (Table 4). The value of qMic has innumerous times been utilized as indicator of soil organic matter capacity to support soil microbial biomass (INSAM; DOMSCH 1988; KASCHUK *et al*., 2010; KASCHUK *et al*., 2011), but it is also possible that microbes in horticultural areas are growing and consuming TOC faster than in native areas due to increased availability of other soil nutrients. In the case, native areas contained more TOC and TN (Table 3) and accumulated larger percentage of oP (Fig. 3) whereas horticultural areas accumulated larger percentage of iP (Fig. 3) within the soil P fractions. These results agree with the fact that P mineralization from soil organic matter can be driven by microbial need for carbon (SPOHN; KUZYAKOV, 2013).

Having intensive agriculture in metropolitan region offers several opportunities for economical growth, but it may also threaten the environment. Therefore, while P limitation is an issue over the world, in metropolitan regions like MRC, excessive P addition may become a potential environmental issue. Intensive agriculture may result in soil erosion (RAMOS *et al*., 2014). This study showed that changes in the soil P fractions suggested a shift in the soil community bacterial structure and in the values of soil microbial biomass of the two different soil ecosystems. The excessive P addition may stimulate soil microbial attack to soil organic matter reserves, which may have consequences for maintenance of soil quality and agriculture sustainability.

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