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RESEARCH ARTICLE

High doses of polypropylene and polyvinyl chloride microplastics affect the microbial community and nutrient status of vineyard soils

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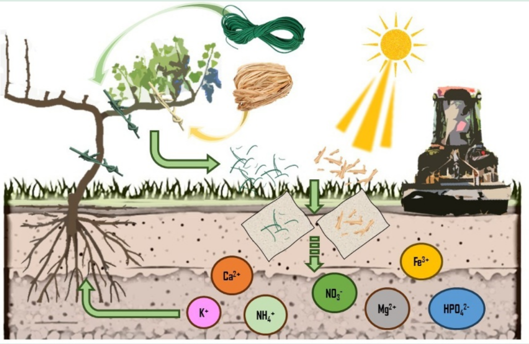
HIGHLIGHTS

- Viticultural string PVC MPs increased soil pH in the acid soil.
- PVC MPs disrupt soil microbes and reduce availability of nitrate by 98%.
- Calcareous soils showed greater resilience to MPs impacts than acidic soils.
- Nutrient availability dropped by 10% for NH_4^+, P, K, Mg, Cu, and 30% for Fe, Mn, Zn.
- Microplastics from used strings have greater impact than new polymers.



ABSTRACT: The escalating use of plastic materials in viticulture causes release of microplastics (MPs) into vineyard soils. This study examines the impact on soil health of polypropylene (PP) raffia and polyvinyl chloride (PVC) tube strings, commonly mulched into the topsoil after use. A 120-d incubation experiment was conducted with soils exposed to high doses (10 g/kg) of microplastics (MPs) from standard, new and used strings. The study investigated alterations in the microbial community, bioavailability of macronutrients (NH_4^+ and NO_3^-, P, K, Ca, Mg), and bioavailability of micronutrients (Cu, Zn, Fe, Mg). The presence of MPs significantly stressed the soil microbial community, reducing microbial biomass by 30% after 30 d, with the exception of PVC in acid soil, which caused an unexpected increase of about 60%. The metabolic quotient (qCO_2) doubled in MP-polluted soils, with PVC exerting a more pronounced effect than PP. Basal respiration increased by 25% relative to the acid control soil. PVC MPs raised soil pH from 6.2 to 7.2 and firmly reduced the bioavailability of micronutrients, particularly in acidic soils, and led to a 98% reduction in nitrate (NO_3^-). The availability of NH_4^+, P, K, Mg decreased by 10% and Cu, Fe, Mn, Zn by 30%. However, Ca availability increased by 30%, despite shifting from the acid-soluble fraction to soil organic matter and crystalline minerals. Calcareous soil was generally more resilient to changes than the acid soil. These findings underscore the urgent need to investigate the long-term effects of MPs from viticulture on soil properties and health.

KEYWORDS: Microplastics, Soil, Nutrient bioavailability, Microbial community, Viticulture

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1 Introduction

It is estimated that 32.3 Mt of plastic waste were produced in Europe, of which about 23% ended up in landfills or in the natural environment (PlasticsEurope, 2023). The consumption of plastics in agriculture amounts to 6.7 Mt/a, because of desirable properties of plastics such as lightness, strength, versatility of use and low-cost (Dar et al., 2022). These advantages also favor the use of plastic products in viticulture, e.g., as inorganic mulch films and protective nets, irrigation pipes, strings, etc. (Vox et al., 2016). In general, winegrowers are aware of their direct actions of introducing plastic materials into the vineyard, but plastics can sometimes be inadvertently introduced into vineyard soil with the application of biowaste (compost, sewage sludge, digestate) (Colombini et al., 2022) or with slow release mineral fertilisers that contain microplastics (MPs) as coating materials (ECHA, 2019). The most common type of plastic found in agricultural soils is polyethylene (PE), followed by polypropylene (PP) and polyvinyl chloride (PVC) (Yu et al., 2020; Kim et al., 2021a; Jia et al., 2022; Khan et al., 2023a; Zhou et al., 2023). The abundance of MPs in agricultural soils ranges from a few particles (Cao et al., 2021) to 105 particles/kg of dry soil (Jia et al., 2022), but specifically in vineyard soils, even higher MPs ranges of 400 to 13,000 particles/kg of dry soil have been observed (Klaus et al., 2024). Since microplastic particles are found in almost all agricultural soils, it is crucial to understand their impact on soil properties and microbial activities, especially in vineyard soils where MPs abundance is notably high.

Previous studies confirmed that MPs can affect soil physical properties such as soil structure, porosity and bulk density (Wang et al., 2022). Similar results have also been reported for soil chemical properties such as increased pH (Zhao et al., 2021), soil organic matter (Feng et al., 2022) and decreased soil nutrient availability (Huang et al., 2022). However, depending on the type, size or shape of the plastic, their effects can be negative, neutral or positive (Wang et al., 2023). Focusing on the effects of PVC and PP MPs on soil, research indicates that these materials can enhance soil quality in several ways. For instance, PVC and PP MPs have been associated with increased cation exchange capacity (CEC) and organic carbon (TOC) content, and reduced soil bulk density (Li et al., 2022; Yu et al., 2023). However, numerous researchers worldwide have warned that MPs are becoming a growing global problem with many negative impacts on the soil. The same has been reported for PVC and PP microplastic particles in soil, where the presence of low concentrations significantly reduces available phosphorus (P) (Yan et al., 2021; Li and Liu, 2022) and significantly reduces nitrate (NO_3^-) levels and increases ammonium (NH_4^+) levels (Shen et al., 2023). In recent years, changes in soil nitrogen cycling and available nitrogen content due to MPs pollution have been documented and have become important topics in soil microplastic research (Lozano et al., 2021; Rong et al., 2021; Li and Liu, 2022; Riveros et al., 2022; Shen et al., 2023). The bioavailability of other macronutrients such as potassium (K), calcium (Ca) or magnesium (Mg), and micronutrients from soil has rarely been studied. However, these nutrients are crucial for grapevine growth, productivity, fruit quality, and overall vine quality and yield (White, 2003).

Understanding the functioning of soil microorganisms is crucial for predicting the biogeochemical cycles of macronutrients and micronutrients. Microplastics in soil can significantly impact microbial communities and nutrient cycling (Khan et al., 2023b). MPs can alter soil microbial diversity, structure, and abundance (Liang et al., 2023; Aralappanavar et al., 2024). Specifically, it has been found that PP MPs can reduce microbial biomass (Blöcker et al., 2020), but do not significantly alter microbial respiration or community composition (Liu et al., 2023). On the other hand, PVC MPs significantly affect agricultural soil microbial activity and community parameters, such as soil respiration (CO_2 emission) and FDA activity (Barili et al., 2023). The presence of PVC MPs also selectively affects the abundances of different soil bacterial and fungal taxa, both at the phylum and genus level (Barili et al., 2023). Many studies on the phytotoxic effects of PVC microplastics in soil have found that PVC MPs exhibit a stronger toxic effect on plants than other plastic materials (Pignattelli et al., 2020; Colzi et al., 2022; Esterhuizen and Kim, 2022) and it is also expected to have significant effects on vineyard microbes.

MPs possess hydrophobic surfaces, large surface areas, and rigid organic structures, enabling them to adsorb nutrients from organic and inorganic fertilisers (Tourinho et al., 2019). Although research on their impact on soil nutrient cycling is nascent (Rillig, 2018), MPs affect soil properties and indirectly influence soil microorganisms and nutrient bioavailability.

This study aims to assess the effects of PVC and PP microplastics from viticulture on soil microbial basal respiration (BR), microbial biomass (GIR), metabolic quotient (qCO_2), and microbial functional diversity (H’), which are crucial for understanding nutrient cycling and availability in vineyard soils. To the best of our knowledge, this is the first study evaluating the impact of new and aged PP and PVC MPs on the availability of N, P, and other macronutrients such as K, Ca, Mg, and micro nutrients (Fe, Cu, Mn, Zn) crucial for vine growth and productivity.

2 Materials and methods

2.1 Study region and soil sampling

Slovenia is a wine-growing country with a total of 15,000 ha of vineyards (SiStat, 2024). There are three wine-growing regions in Slovenia; in our study we sampled vineyard soils in the Primorje and Podravje regions. These two wine-growing regions were selected due to the significant use of synthetic polypropylene (PP) raffia and polyvinyl chloride (PVC) tube strings by growers. It is common to use 2–5 strings, equivalent to about 2.4–6.0 g of plastic, to tie each vine to a wooden stake. We may assume that about 30% of the plastic strings (the lower part of the support) is damaged every year through mechanical handling. This means that on a hectare of vineyard with 5,000 vines, up to 9 kg of plastics may be introduced into the soil each year. Consequently, a higher level of MPs pollution is predicted in Slovenian vineyards in the next decade.

Two soils of contrasting pH were selected. The acid soil was sampled in Podravje (Stajerska) in a wine growing region in NE Slovenia and the calcareous soil in Primorje (Vipava Valley) in a wine growing region in SW Slovenia. Soil samples were classified as Dystric Gleysol (acid soil sample) and Eutric Cambisol (calcareous soil sample) according to the Slovenian soil classification (Prus et al., 2015). Both vineyards were more than 20 years old and were selected because no plastic have been used before. Sampling was done with an auger at 20–25 different points randomly distributed throughout the vineyard to a depth of 20 cm. The approximate mass of the composite sample taken on the field was about 20 kg.

The soil samples underwent sieving (2 mm) and 0.5 kg was set aside for air-drying for chemical analyses (Table 1), while the remainder was kept moist at 5 °C temperature and used for incubations. Part of the air-dry soil sample was additionally ground and sieved through a 250 μm sieve for measurement of total nutrients and metals.

2.2 Microplastic materials and types

Virgin polypropylene (PP) (Sigma Aldrich CAS No. 9003-07-0) in granular form and polyvinyl chloride (PVC) (Sigma Aldrich CAS No. 9002-86-2) in powder form were used as standard control and referred to as standard micro-PP/PVC in the text. New polypropylene (PP) vineyard string (synthetic vineyard raffia) and new polyvinyl chloride (PVC) vineyard tube string were purchased from a local vitioenocultural supplier and are here referred to as new micro-PP/PVC in the text. Used polypropylene (PP) vineyard raffia and polyvinyl chloride (PVC) vineyard string pieces (here referred to as used micro-PP/PVC) were collected from the same vineyards where the soil samples were taken. The PP raffia had been in the vineyard for one year, as it had been used to tie up the one-year-old vine sprouts, and the PVC strings had been in the vineyard from 1 to more than 10 years, as they were used to tie up the vines to the holder.

2.3 Microplastic grinding and soil spiking

Fine particles of each material were obtained by grinding the coarse fractions: after cooling (6 min) in liquid nitrogen (LN) (Messer, Germany), four rounds of grinding for 3 min at 30 Hz were carried out with a Retsch zirconia ball mill with intermediate cooling in LN for 1 min between each round. The ground parti-

Table 1 Main physico-chemical properties of soils

Soil property	Soil type	
	Calcareous soil	Acid soil
pH	7.74 ± 0.08	6.07 ± 0.06
Carbonates (g/kg)	118.7	8.2
TOC (g/kg)	2.70	2.10
TON (g/kg)	0.22	0.21
C:N	12.5	10.1
Olsen P (mg/kg)	48.58 ± 2.07	48.57 ± 10.15
NO_3^-–N (mg/kg)	32.51 ± 0.98	44.74 ± 2.88
NH_4^+–N (mg/kg)	9.65 ± 0.44	5.86 ± 0.52
K total (mg/kg)	5645 ± 2804	6415 ± 330
Ca total (mg/kg)	6698 ± 554	3290 ± 202
Mg total (mg/kg)	5469 ± 969	8160 ± 451
Fe total (mg/kg)	42424 ± 3314	48358 ± 2586
Mn total (mg/kg)	1809 ± 44	842 ± 310
Cu total (mg/kg)	129.3 ± 0.7	178.2 ± 4.2
Zn total (mg/kg)	149.4 ± 13.1	184.7 ± 13.7
Texture category	Silt loam	Loam
Sand (g/kg)	260	420
Silt (g/kg)	510	410
Clay (g/kg)	230	170

cles were sieved through 5 mm stainless steel sieves to obtain microplastic particles less than 5 mm in size following the general terminology of MPs (Frias and Nash, 2019). The size distribution of the PVC and PP MPs was measured in 5 g of a well-mixed MPs subsample: 1% of the particles were between 5 and 2 mm, 10% were between 2 and 1 mm, 70% were between 1 mm and 250 µm, and 20% were less than 250 µm. The microplastic particles were irregularly shaped as fragments (Fig. S1).

Before spiking with MPs, the acid and calcareous soils were pre-incubated aerobically for 14 d at 25 °C and 40% water holding capacity. The soils were mixed subsequently with each type of ground microplastic at a concentration of 1.0% (w/w), simulating elevated microplastic pollution levels (see Supplementary Materials for details). This equated to 20 g of PP and PVC MPs being stirred manually into 2 kg of soil for 15 min. The control soil was stirred in the same way as the samples spiked with MPs to create the same level of disturbance.

2.4 Experimental design and setup

The experiment was conducted under laboratory settings at the Wine Research Centre (Vipava, Slovenia), and followed a three factors design including “soil type”, “plastic material”, and “plastic type”. The factor “soil type” had two levels: acid or calcareous; the factor “plastic material” had two levels: polyvinyl chloride (PVC) and polypropylene (PP) and the third factor “plastic type” had three levels: standard, new, and used. Acid and calcareous soils with no plastic addition were used as controls. Soil samples were maintained at a constant temperature of 25 °C for 120 d in a 10-L container with four gas exchange vents in darkness. To maintain soil moisture at ~40% water-holding capacity throughout the incubation period, we added distilled water to the pots every seven days according to the weight loss due to evaporation.

Physical and chemical soil analyses were conducted at the beginning and end of the incubation period. Additional subsampling was performed every 30 d to analyze microbiological changes in the soil.

2.5 Analytical methods

2.5.1 Physiologic variables and soil microbial community

The MicroResp™ method developed by Campbell et al. (2003) was used to assess changes in microbial activity after addition of MPs. CO_2 evolution was assessed at 30-d intervals according to the substrate-induced respiration (SIR) system described in the manufacturer’s guidelines. A range of 14 different C-substrates (at 30 mg C/g soil) were added: four carbohydrates (L-arabinose, D-fructose, D-glucose, and D-trehalose), six amino acids (L-alanine, L-arginine, L-cysteine HCl, γ-amino butyric acid, L-lysine, and N-acetyl glucosamine) and four carboxylic acids (citric acid, L-malic acid, oxalic acid and α-ketoglutaric acid). To quantify basal respiration (BR), and microbial biomass, expressed as Glucose Induced Respiration (GIR), pure water and D-glucose (at 30 mg C/g soil) was added (Anderson and Domsch, 1978). After six hours of incubation at 25 °C, the color change was measured with a microplate reader (Infinite, TECAN Instruments, Crailsheim, Germany) at 590 nm. The CO_2 production rates were expressed in units of µg CO_2-C/(h·g) of soil.

The metabolic quotient for CO_2 (qCO_2) was calculated according to the equation proposed by Anderson and Domsch (1993): qCO_2 = basal respiration (BR) / microbial biomass (GIR). Moreover, microbial functional diversity (H’) was determined using the Shannon–Weaver index (H’): $H' = -\Sigma\ Pi\ (\ln Pi)$, where Pi is the ratio of the activity of a particular substrate and the sum of the activities of all 15 substrates used in the study (Zak et al., 1994).

2.5.2 Soil physico-chemical properties and bioavailability of nutrients

The two soils were characterized for texture, pH, total carbonates, total organic carbon (TOC), total nitrogen (TN), available phosphorus (Olsen P) and inorganic nitrogen (NO_3^- and NH_4^+). Soil texture was measured in a Bouyoucos cylinder with an ASTM 152H hydrometer. Soil pH was measured in water (1:2.5, weight-to-volume ratio). Total organic carbon and total nitrogen (TOC and TN) were determined in an elemental analyzer by automated thermal analyses (Costech Instruments). Carbonates were previously removed by treatment with HCl. C/N ratios were then calculated. Inorganic C analysis (carbonates) was determined using a modified pressure calcimeter method. Olsen P was measured using 2 g of soil sample extracted with 40 mL of 0.5 mol/L $NaHCO_3$ (Olsen, 1954). Nitrate (NO_3^-) and ammonium (NH_4^+) were measured after extraction of 5 g of soil with 25 mL of 0.5 mol/L KCl (Mulvaney, 1996). The extraction solution was filtrated and NO_3^- and NH_4^+ determined by a segmented flow injection analyzer (Skalar San^{++}, NL).

Pseudo-total nutrient and metal concentrations in soil were measured using the USEPA 3052 (USEPA, 1995) mineralisation method and Inductively Coupled Plasma

Atomic Emission Spectroscopy, (ICP-AES) using an Agilent 5800 instrument (Agilent). Bioavailable cationic macronutrients (i.e., Ca, Mg, K) were analyzed by extraction with a 1 M $BaCl_2$ solution, whereas micronutrients (Cu, Zn, Fe and Mn) were analyzed with the DTPA/TEA method developed by Lindsay and Norvell (1978). A certified soil (BCR–CRM 600) was also extracted to ensure quality control of the analyses. Moreover, potential bioavailability of nutrients and metals was also evaluated using two single-step extractions proposed by Vázquez et al. (2016) for vine plants. The extraction solutions were analyzed by ICP-AES (Agilent 5800). Calibration was performed using standard solutions (0.5, 1, 5, 10, 30, and 50 mg/L) prepared from an ICP-standard 23-element solution in 5% HNO_3 (Merck solution IV), with yttrium (Y) as the internal standard. Strict quality assurance and quality control (QA/QC) were carried out including blanks, analysis of reference material (CRM 601), and analysis of laboratory control samples.

2.5.3 Nutrient and metal fractionation

The distribution of nutrients and metals in the soil solid phase was determined in triplicate using the four-step sequential extraction scheme proposed by the Community Bureau of Reference (BCR) method (Rauret et al., 1999).

2.6 Statistical analysis

Measurements and analyses on soils were based on oven-dried soil, replicated three times and reported as mean ± standard deviation (SD). Microbial biomass and Shannon-Weaver index data were normally distributed, but basal respiration data were normalized and the natural log-transformed before statistical analysis. Data were analyzed using analysis of variance (ANOVA) with the Tukey HSD post-hoc test. Differences between treatments were considered significant at $p < 0.05$ and identified in the Figures and Tables with different letters. All descriptive statistical analyses were performed using the R statistical program (R Development Core Team, 2018).

3 Results and discussion

3.1 Microbial parameters

The effects of MPs on the soil microbial community was assessed at 30-d intervals from the first day of incubation.

It was observed that the basal respiration levels in soils containing micro-PVC were consistently larger (from 10% to two times higher) relative to control samples and soils with micro-PP, at all sampling times, (Figs. 1(c) and 1(d)). Conversely, micro-PP contaminated soils did not differ substantially from controls. One possible reason for this result could be the utilization of micro-PVC particles by microbes, similar to the findings of Barili et al. (2023), who observed a reduction in the number and size of PVC particles over a one-year experiment due to microbial degradation. Additionally, soil respiration is closely correlated with soil organic matter, which serves as a primary source of energy and nutrients for soil microorganisms that drive the process of soil respiration. Increased soil organic matter content generally enhances microbial activity, thereby resulting in elevated rates of soil respiration (Wang et al., 2003). In our study, soil organic matter, as indicated by total organic carbon (TOC), exhibited a notable increase of nearly 50% in samples containing micro-PVC particles, compared with a modest 7% increase observed in samples with micro-PP particles at the end of the incubation period. This effect was measurable in both calcareous and acid soil samples (Table 2). It should be noted that none of the current methods for assessing soil carbon content are routinely able to distinguish soil organic C from C contained in MPs, because the MP quantification protocols are still under development (Kim et al., 2021b). However, synthetic MPs are known to be substantially comprised of carbon, typically constituting around 80% of their composition (Rillig, 2018). Carbon bound within synthetic materials may serve as a carbon source for bacterial strains isolated from environmental samples, as demonstrated in studies investigating synthetic PVC microplastic particles (Patil and Bagde, 2012) and other MPs (Zhang et al., 2022). MPs enable the bacteria to migrate and colonise at the plastisphere (Wang et al., 2023), where biofilms are formed with higher biomass on plastics compared to natural substrates (Miao et al., 2020). Consequently, the observed augmentation in soil organic matter induced by the carbon content of synthetic MPs might explain the notable increase in basal soil respiration observed in soil samples contaminated with micro-PVC particles.

Despite the much higher basal respiration in the micro-PVC contaminated soil samples, we found that the microbes in these samples required more substrate and energy for their metabolic processes (Awet et al., 2018; Zhang et al., 2022). This can be confirmed by the metabolic quotient (qCO_2) results, because qCO_2 was significantly higher in the samples contaminated with

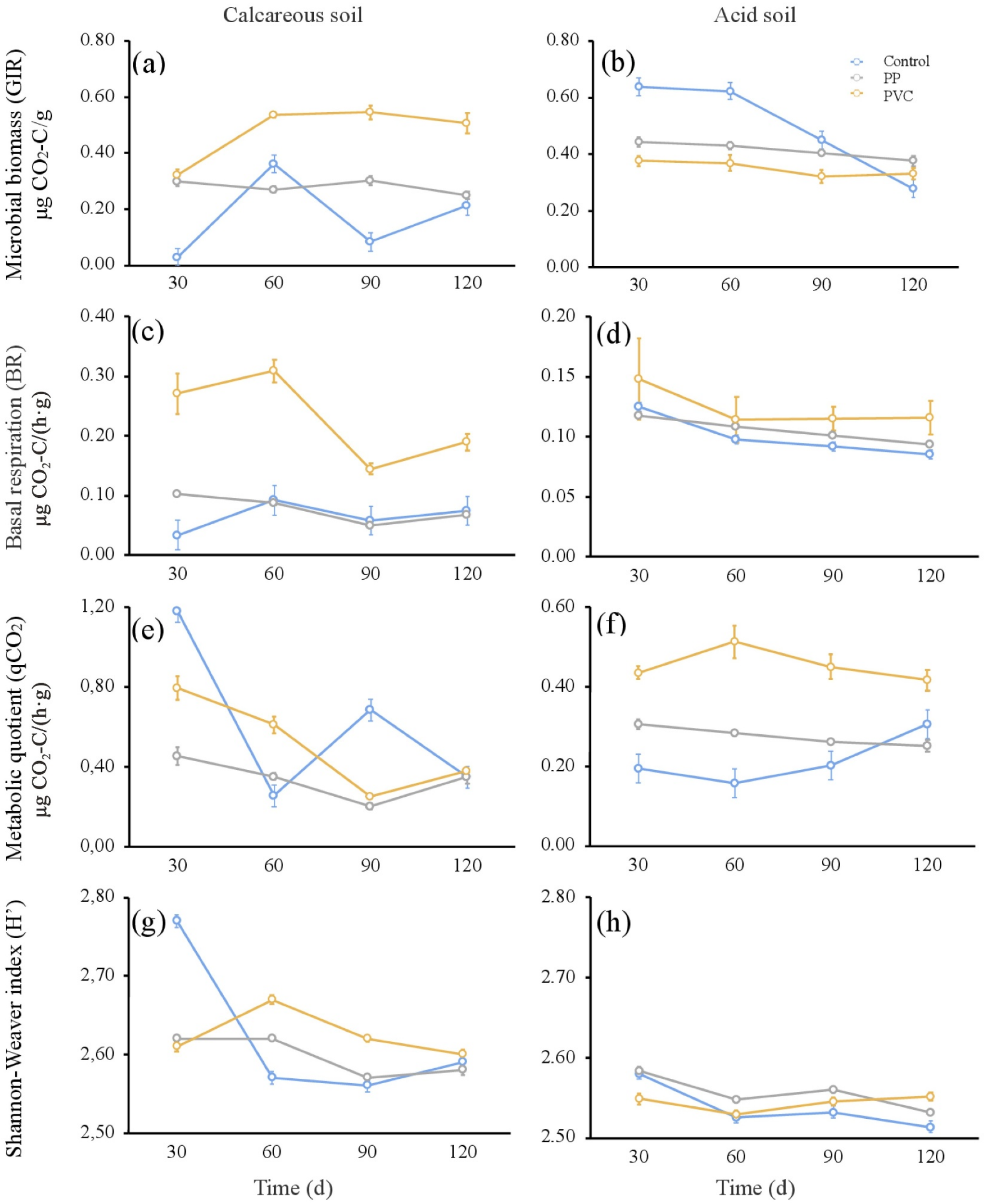


Fig. 1 Effect of PP and PVC microplastics on soil microbial biomas (GIR), basal respiration (BR), metabolic quotient (qCO_2) and microbial diversity, Shannon-Weaver index (H') obtained from the SIR bioassays in calcareous and acid soil after 120 d of incubation. Control are soils without MPs. Error bars represent standard error of three replicates.

micro-PVC plastic particles compared to the soil containing micro-PP and the control (Figs. 1(e) and (f)). A high qCO_2 for a microbial community may indicate a shift toward the use of synthetic substrates or a change in physiologic status due to stress caused by MPs in soil, similar to the response observed under other stress conditions (Szili-Kovács and Takács, 2024). Analogous to the BR, qCO_2 was also its highest when micro-PVC particles derived from the used PVC tube ties were introduced into the soil (Fig. S2).

In the acid soil, the microbial biomass (GIR) reached its highest level after 60 d of incubation (0.54 ± 0.01 μg C/g dry soil), particularly evident in the soil treated with micro-PVC particles (Fig. 1(b)). Conversely, in the calcareous soil, the microbial biomass had already generally peaked after 30 d and decreased thereafter, with the highest peak observed in the soil treated with PP (0.44 ± 0.02 μg C/g dry soil) (Fig. 1(a)). Subsequen-

Table 2 Soil pH, total organic carbon (TOC) and bioavailability of micronutrients in vineyard soils after 120 d of incubation for control (no MPs) and MPs polluted calcareous and acid soil

Soil type	Treatment	pH	TOC (g/kg)	Available micronutrients (mg/kg)			
				Cu	Zn	Fe	Mn
Calcareous soil	Control	$^{b}7.67 \pm 0.04^{a}$	27.0	$^{a}33.16 \pm 1.22^{a}$	$^{a}4.00 \pm 0.18^{a}$	$^{a}55.86 \pm 0.76^{a}$	$^{a}63.07 \pm 1.02^{a}$
	PP new	7.66 ± 0.10^{a}	23.0	28.97 ± 1.41^{b}	3.89 ± 0.12^{a}	46.06 ± 1.74^{b}	56.20 ± 2.30^{a}
	PP used	7.51 ± 0.16^{a}	29.0	29.32 ± 1.37^{b}	4.13 ± 0.14^{a}	46.93 ± 4.09^{b}	57.08 ± 6.00^{a}
	PP std	7.57 ± 0.03^{a}	37.0	29.88 ± 1.09^{b}	4.11 ± 0.06^{a}	48.85 ± 0.63^{b}	60.28 ± 2.12^{a}
	PVC new	$^{a}7.79 \pm 0.00$	57.0	$^{b}30.50 \pm 0.65$	$^{a}3.85 \pm 0.04$	$^{b}50.66 \pm 0.51$	$^{b}59.06 \pm 0.46$
	PVC used	$^{a}7.77 \pm 0.02$	60.0	$^{b}30.17 \pm 7.7$	$^{a}4.06 \pm 0.13$	$^{b}49.64 \pm 2.17$	$^{b}56.85 \pm 2.04$
	PVC std	$^{c}7.54 \pm 0.00$	24.0	$^{b}29.39 \pm 0.56$	$^{b}3.27 \pm 0.03$	$^{b}51.62 \pm 1.20$	$^{b}58.87 \pm 0.89$
Acid soil	Control	$^{c}6.19 \pm 0.02^{a}$	21.0	$^{a}49.09 \pm 0.75^{a}$	$^{a}7.23 \pm 0.17^{b}$	$^{a}83.62 \pm 1.66^{a}$	$^{a}45.30 \pm 1.50^{a}$
	PP new	6.10 ± 0.01^{b}	27.0	48.12 ± 1.57^{a}	7.85 ± 0.31^{a}	73.75 ± 7.48^{ab}	37.63 ± 5.22^{ab}
	PP used	6.07 ± 0.01^{b}	33.0	47.77 ± 0.89^{a}	7.60 ± 0.04^{ab}	68.82 ± 1.17^{b}	34.21 ± 3.88^{b}
	PP std	6.10 ± 0.02^{b}	34.0	47.72 ± 2.10^{a}	7.68 ± 0.43^{ab}	74.17 ± 8.94^{ab}	37.82 ± 6.49^{ab}
	PVC new	$^{a}7.16 \pm 0.01$	34.0	$^{bc}41.71 \pm 0.27$	$^{b}5.80 \pm 0.10$	$^{c}48.62 \pm 0.12$	$^{b}18.20 \pm 0.08$
	PVC used	$^{b}7.07 \pm 0.03$	47.0	$^{c}40.50 \pm 1.74$	$^{bc}5.69 \pm 0.26$	$^{bc}54.18 \pm 10.33$	$^{b}24.20 \pm 14.52$
	PVC std	$^{c}6.21 \pm 0.01$	58.0	$^{b}42.97 \pm 0.57$	$^{c}5.41 \pm 0.08$	$^{b}63.88 \pm 1.03$	$^{b}31.10 \pm 0.29$

*Footnote: All data are expressed as average value ± standard deviation ($n = 3$).

^{a,b,c} Different letters within the same plastic type (compared to control) indicate significant differences (Tukey test; $p < 0.05$). Letters in the left indicate differences for micro-PVC and letters on the right indicate differences for micro-PP particles.

Grained PVC and PP ties were added to the soil: new ties purchased at a local shop (PVC/PP new), previously used ties from the sampled vineyard (PVC/PP used) and polyvinyl chloride and polypropylene pure polymers (PVC/PP std).

tly, the microbial biomass in the soil samples containing MP particles remained consistently high until the end of the incubation period, whereas in the control soil samples GIR halved. This result implies that soil microorganisms could adapt to the plastic pollution in a relatively short time and utilize the synthetic C source present in MPs, as we did not add any other C source during the incubation period. In the control samples without MPs, in which the soil microorganisms had consumed most of the C source after 60 d of incubation, the microbial biomass declined gradually relative to control. This outcome is also supported by the results of the Shannon-Weaver index (H’), which indicated that the community functional diversity in control soils simplified significantly after 60 d of incubation compared to the MP-polluted soil samples. The H’ values from micro-PP and micro-PVC polluted samples were 2% and 4% higher, respectively, relative to control soils (Figs. 1(g) and 1(h)). The highest diversity index was measured in soil samples spiked with microplastics from used PVC plastic strings (Fig. S2). The plastic strings collected from the vineyards prior to the incubation experiment were not sterilised because we wanted to understand whether the indigenous soil microorganism community present on the old ties influenced the diversity of soil microorganisms, and how this differed from the presence of MPs from new ties. Based on the results presented in Fig. S2 we can confirm this hypothesis: the Shannon diversity index in soil samples containing MPs from used PVC vineyard ties was the highest.

3.2 Bioavailability of macronutrients

3.2.1 Bioavailability of nitrogen

Available nitrogen (N) content was measured in terms of NH_4^+ and NO_3^- concentrations. The results are presented in Fig. 2. In calcareous soils, the NH_4^+content was reduced by 31% due to the presence of micro-PP particles, but in acid soils, micro-PP did not change the NH_4^+ content in the soil compared to the control soils without added plastics. On the other hand, the presence of micro-PVC particles in calcareous and acid soils reduced NH_4^+ content by 21% and 23%, respectively. These results do not agree with previous studies where the presence of micro-PVC particles had no significant effect or even increased the NH_4^+ content in the amended soils (Liu et al., 2017; Yan et al., 2021). The difference between this research and previously published results could be related to the different concentrations of MPs used or to the experimental conditions employed. Furthermore, PVC microplastic may form biofilms that lead to NH_4^+ fixation by

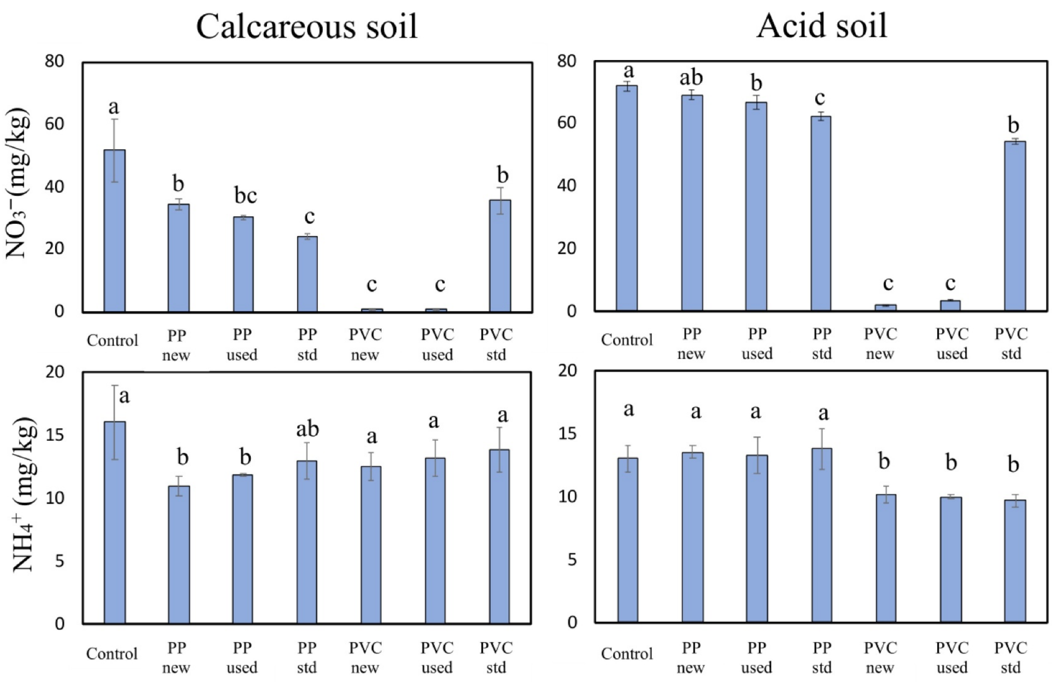


Fig. 2 Nitrate (NO_3^-) and ammonium (NH_4^+) content in calcareous and acid soils after 120 d of incubation in control (no MPs) and MPs contaminated soils. Grained PVC and PP ties were added to the soil: new ties purchased at viti-oenological local shop (PVC/PP new), previously used ties from the sampled vineyard (PVC/PP used) and polyvinyl chloride and polypropylene pure polymers (PVC/PP std). Error bars represent standard error of three replicates. Different letters denote significant differences between treatments within each soil type according to Tukey's multiple comparison test ($p < 0.05$).

microbes (Chen et al., 2020), which in the long-term can result in lower levels of NH_4^+ in the soil. In general, our results showed that soil NO_3^- levels were affected more than NH_4^+ levels, with the presence of microparticles leading to a reduction in NO_3^- content in the soil compared to the control without the addition of plastic particles (Fig. 2).

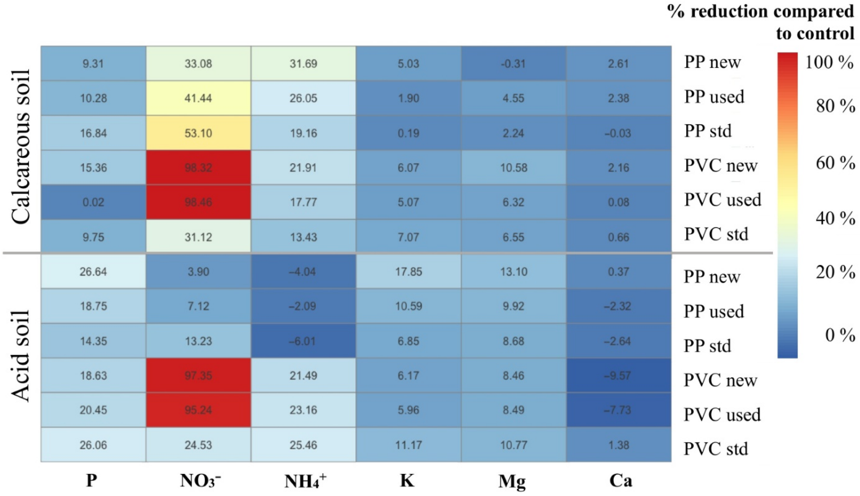
A significant reduction in soil NO_3^- content (up to 50%) was observed when micro-PP particles were present in calcareous soil and NO_3^- depletion was measured in micro-PVC-spiked calcareous and acid soils (soil available NO_3^- content was 0.8 and 3.5 mg/kg, respectively). Interestingly, this effect of NO_3^- depletion in the soil was only observed in the soils with micro-PVC particles from viticulture strings, but not in the soils where the standard micro-PVC was spiked. In these cases, the decrease in available NO_3^- content was 24% in acid soils and 30% in calcareous soils. In a study by Yan et al. (2021), calcareous and acid soils were also spiked with micro-PVC particles, and the decrease in NO_3^- (by 10%) was found in calcareous soils, but the experimental period was only 35 d. A similar decrease in NO_3^- content was also measured in calcareous soils in a study by Liu et al. (2017) where the time-related decrease in soil available NO_3^- was detected. They determined that the decrease in available soil NO_3^- with micro-PP particles was greatest in the first three days after the addition of the plastic and did not differ significantly later.

Several studies have reported that the addition of MPs to the soil affected the nitrogen cycle at different levels by altering the microbiota and the abundance of genes, and thus the enzymes that catalyze the different stages of the nitrogen cycle (Riveros et al., 2022). Based on our results, there are several possible explanations for the NO_3^- reduction in the soil. The first, but least plausible explanation, is that the denitrification process is promoted by *Pseudomonaceae.* These bacteria (which belong to the *Proteobacteria)* have the ability to promote both nitrification and denitrification in the soil. As denitrification is primarily carried out by facultative anaerobic bacteria that normally respire oxygen (O_2), but in its absence also respire N oxides (Groffman, 2012). The concentration of 1.0% (w/w) of micro-PVC particles in soil is a high dose and can lead to local anoxic conditions, confirmed also by the high basal respiration, where facultative anaerobic bacteria already start denitrification. It has been reported elsewhere that the abundance of *Pseudomonaceae* in loamy and sandy soils increases with the addition of micro-PVC (1% and 5% w/w) (Fei et al., 2020; Yi et al., 2021) so denitrification may also promote soil NO_3^- reduction. Although we do not have sufficient evidence, we hypothesize that the micro-PVC particles in the present study most likely inhibited the nitrification process, which in turn hindered the oxidation of NH_4^+ and led to a decrease in NO_3^-. This was also found by Sun et al. (2020) in the composting of cow manure, where NO_3^- levels were much lower in the presence of PVC microplastics than in the presence of other microplastics. An inhibited nitrification process in the soil could be the reason why NO_3^- levels decreased significantly after the addition of standard micro-PVC particles. However, it is not yet known why the addition of micro-PVC particles causes NO_3^- depletion in the soil. Further studies are needed, especially with regard to the additives and plasticisers (which are mostly trade secrets) present in the commercial PVC tube strings.

3.2.2 Bioavailability of phosphorus

The availability of phosphorus (Olsen P) in soils without microplastics increased after 120 d of incubation for both calcareous and acid soils (Table S1). However, the bioavailability of Olsen P in soils with microplastics decreased; acid soil samples with microplastic (average decrease of 20%) were more affected than calcareous soil samples (average decrease of 10%) for both types of amended microplastics (micro-PP and micro-PVC) (Fig. 3). First, the decrease in soil P content after microplastic contamination could be related to the change in soil aggregate size, leading to a decrease in soil bulk density, as already found in other studies (de Souza Machado et al., 2019; Chia et al., 2022). On the other hand, we can see from the previously presented results that the microbial biomass (GIR) and the Shannon-Weaver diversity index (H’) in soil contaminated with MPs remained high until the end of incubation compared to the control soil. The higher the biological activity, the more P was taken up by the soil microorganisms to fulfil their anabolic needs. It has been shown that a higher content of polypropylene (PP) MPs in the first 7 d after addition to the soil significantly increases the dissolved organic P content due to the function of microbe-mediated solubilisation of inorganic P and mineralisation of organic P (Liu et al., 2017), and similar effects may be expected also for PVC. In the long-term, this may lead to a reduction in P availability and it may also be the reason for the reduction in availability due to the presence of micro-PVC particles. Our finding that micro-PVC plastics cause a reduction in P availability is also reflected in the study of Li and Liu (2022), where a similar experimental design was conducted.

Although we found a clear difference in NO_3^- availability between the PVC plastics used and the standard PVC, this difference was not observed for Olsen P. The availability of Olsen P decreased in the presence of all of the MPs particles tested. The lowest impact was observed for used vineyard strings in calcareous soils and the greatest decrease in



		P	NO_3^-	NH_4^+	K	Mg	Ca
Calcareous soil	PP new	9.31	33.08	31.69	5.03	-0.31	2.61
	PP used	10.28	41.44	26.05	1.90	4.55	2.38
	PP std	16.84	53.10	19.16	0.19	2.24	-0.03
	PVC new	15.36	98.32	21.91	6.07	10.58	2.16
	PVC used	0.02	98.46	17.77	5.07	6.32	0.08
	PVC std	9.75	31.12	13.43	7.07	6.55	0.66
Acid soil	PP new	26.64	3.90	-4.04	17.85	13.10	0.37
	PP used	18.75	7.12	-2.09	10.59	9.92	-2.32
	PP std	14.35	13.23	-6.01	6.85	8.68	-2.64
	PVC new	18.63	97.35	21.49	6.17	8.46	-9.57
	PVC used	20.45	95.24	23.16	5.96	8.49	-7.73
	PVC std	26.06	24.53	25.46	11.17	10.77	1.38

Fig. 3 Heatmap is showing the changes in macronutrient bioavailability (%) in MPs spiked calcareous and acid soils compared to control soils (no MPs). Positive values represent a decrease in bioavailability and negative values represent an increase in nutrient bioavailability. Grained PVC and PP ties were added to the soil: new ties purchased at viti-oenological local shop (PVC/PP new), previously used ties from the sampled vineyard (PVC/PP used) and polyvinyl chloride and polypropylene pure polymers (PVC/PP std).

bioavailability was measured for new PP (PP new) in acid soils (Fig. 3). The results showed that the microplastic pollution from the plastic ties used by the vine growers did not have a significant impact on P availability in the soil.

3.2.3 Bioavailability of potassium, calcium and magnesium

The availability of other macronutrients important for normal vine growth, such as potassium (K), calcium (Ca) and magnesium (Mg), was also affected by the presence of microplastics (Table S1). In calcareous soils, the effect of micro-PVC particles was significant because they reduced the availability of K (an average decrease in availability of up to 7%) and Mg (an average decrease in availability of up to 10%) (Fig. 3). On the other hand, the addition of micro-PVC particles in acid soils did not result in such a significant decrease in K and Mg availability, but there was a greater effect on K and Mg following the addition of micro-PP particles. The availability of K decreased by up to 18% and the Mg availability decreased by up to 13% compared to the control soils. Ca availability was not affected by the presence of micro-PVC particles in calcareous soils, but resulted in a significant increase of up to 10% in acid soils compared to control soils. Again, the influence of the type of plastic tested was important. Micro-PVC particles from used tube strings had significantly greater availability than the control without plastic or standard micro-PVC particles. This may be due to the additives used to optimise the plastic's thermal properties, with PVC production using commercial thermal stabilizers formulated as Ca-based powders (Shnawa et al., 2016). This effect was not measurable in calcareous soils because the binding of Ca^{2+} to organic matter at normal pH is much higher than in soils with lower pH (i.e., acid soils) (Christl, 2012).

To our knowledge, there are few studies that have investigated the availability of macronutrients such as potassium (K), calcium (Ca) or magnesium (Mg) in the presence of microplastics. However, Yang et al. (2021) reported a significant decrease in available K concentration in soil after the addition of micro-GPPS and micro-HDPE particles to soil, but a direct comparison to our results is not recommended due to the great variation in the properties of different MPs. PVC MPs are pollutants with rubbery properties, low crystallinity, and weak acceptors that lower the soil's cation exchange capacity, which is the potential of the soil to release nutrient cations such as Ca^{2+}, Mg^{2+}, and K^+ into the soil solution to be assimilated by plants (Khaledian et al., 2017). This means that only a small number of molecules are able to bind (react) at the soil surface (Chia et al., 2022). Once sorption in the soil decreases after microplastic contamination, adsorption of some heavy metals is promoted, such as Cu in polluted viticultural soils. This impairs the bioavailability of macronutrients to plants and can even cause leaching into groundwater of heavy metals previously bound to the macronutrient complex, polluting the environment (Chen et al., 2021).

3.3 Bioavailability of micronutrients

The availability of micronutrients such as copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn) was also studied (Table 2, Fig. S3). The results indicate that microplastic particles also affect the availability of micronutrients. This effect was most evident in the acid soil and in the presence of micro-PVC particles. The micro-PP particles in the soil did not lead to such a strong decrease in micronutrient availability (on average less than 10%), although the decreases in the bioavailability of almost all micronutrients was statistically significant. In contrast, the statistically significant reductions in the availability of Cu, Zn, Fe, and Mn in the presence of micro-PVC particles were more substantial, with values of up to 17%, 25%, 40%, and 60%, respectively (Fig. S3). Similar reductions in micronutrient bioavailability from soils have been reported previously (Moreno-Jiménez et al., 2022; Liu et al., 2023).

The availability of micronutrients is strongly dependent on the pH of the soil. As the pH increases (Table 2), the micronutrient cations are hydrolysed, which is the first step in the precipitation of insoluble hydroxides. Therefore, Cu, Zn, Fe, and Mn deficiencies could occur in vineyards at a soil pH of > 7 (White, 2003). From our results (Table 2), there were significant increases in pH in treatments where micro-PVC particles were added to calcareous soils (pH from 7.6 to 7.8) and particularly acid soils (an increase in magnitude from pH 6.1 to 7.1), which were not observed in the presence of micro-PP particles. This could be one of the reasons why a larger reduction in micronutrient availability was recorded in acid soils than in calcareous soils.

3.4 Changes in nutrient fractionation

The BCR four-step sequential extraction procedure was performed to evaluate the binding of both macronutrients (Ca, Mg) and micronutrients (Cu, Zn) to different soil fractions. Furthermore, an extraction with EDTA and an acetic acid solution developed by Vázquez et al.

(2016) was also performed. Besides the extraction with $BaCl_2$ solution (for Ca and Mg) and DTPA/TEA (for Cu and Zn), these additional bioavailability extractions were used because the empirical model for predicting nutrient bioavailability in the vineyard-soil-vine-grape system demonstrated a significant relationship between the sum of bioavailable nutrient concentrations from the soil and the level of accumulated nutrients in vine leaves and grapes.

According to the results of sequential extraction (Fig. 4), Ca was the most bioavailable element in both calcareous and acid soils, as most of the Ca (up to 60%) was extracted under mild acid conditions (Step I). In contrast, most of the Mg and Zn were extracted under harsh oxidant acid conditions, and are thus associated with the crystalline lattice of the soil minerals (Step IV). Cu, on the other hand, is mainly associated with Fe and Mn oxides (Step II) and organic matter (Step III). We determined that micro-PVC particles altered the speciation of Ca, Cu, and Zn. Micro-PVC particles also decreased the bioavailability of Ca in both calcareous and acid soils, because the association with the acid-soluble fraction was reduced, and the binding to the soil organic matter and the crystalline lattice of primary and secondary minerals increased. A completely opposite situation was observed for Zn in acid soil, where the association of primary and secondary minerals with the residual fraction of the crystalline lattice decreased, and the association with the acid-soluble fraction increased. In the case of Cu, the association with Fe and Mn oxides decreased and the acid-soluble fraction increased. Fractionation of Mg in soil with micro-PVC particles did not change significantly.

Based on the results of the four-step sequential BCR extraction, we can assume that the bioavailability of Ca in the presence of micro-PVC particles is much lower than in the control soils without plastic. However, we cannot prove this, since the availability of Ca presented in Section 3.2.3 and also measured after acetic acid extraction (by 30%) and after EDTA extraction (by 12%) was higher in calcareous soils and twice as high in acid soils (Fig. 5). However, our expectations for the bioavailable fraction of Zn in acid soil samples were in agreement. Here, the association with an acid-soluble fraction increased, and the bioavailability of Zn in the presence of micro-PVC from soil samples after acetic acid extraction was also significantly higher than in control soils. No significant changes in metal fractionation were measured for Zn in calcareous soils after acetic acid extraction compared with the control soil sample. Similar results were also obtained after EDTA extraction of calcareous soils (Fig. 5), where PVC used particles did not significantly reduce the bioavailability of Zn. This result is contrasts with the results of Yu et al. (2021), who found that the addition of polyethylene microparticles to soil significantly reduced Zn availability, especially in the microaggregate fraction (53–250 μm size). Nevertheless, as mentioned before, it is difficult to compare the effects on soil properties when there are differences in the type, size or shape of microplastic particles used in the experiments (Wang et al., 2023).

Extraction of vineyard soils with EDTA solution generally showed no changes in most nutrients in the MPs-containing soils compared to the control soils without MPs (Fig. 5). Nevertheless, a significant increase in the bioavailability of Ca was measured in the presence of micro-PVC, with increases of 45% in acid soils and 12% in calcareous soils. This increase in Ca bioavailability was expected, since EDTA increases its complexing power with progressive rises in pH (Brown and Elliott, 1992). After the addition of micro-PVC particles, the increase in pH in acid soil samples was of greater magnitude than in calcareous soil samples (Table 2), and this was also the reason for the higher Ca bioavailability in acid soil samples.

A completely different situation was measured for Ca bioavailability in the standard micro-PVC treatment (Sigma Aldrich) and for Mg bioavailability after extraction with EDTA solution, although we would expect the same increase in Ca bioavailability after the addition of micro-PVC particles from used ties. The results from soil incubated with micro-PVC particles of standard plastic showed a significant decrease in Mg bioavailability after 120 d compared to the calcareous control soil. The situation in acid soils was even more coherent. In all treatments (all types of micro-PVC plastic) where microplastic was added, Mg bioavailability decreased significantly (by 93%) compared to the control soil. Since sequential extraction and extraction with acetic acid solution did not show differences in Mg bioavailability, we assume that in EDTA extraction a stoichiometric amount of EDTA was not sufficient for the extraction of the divalent Mg^{2+} ion (Papassiopi et al., 1999). It was mentioned earlier that Ca is used as a PVC additive in the manufacture of plastic products, so the access of Ca in the micro-PVC treatments can be expected (but not with the standard plastic). Because the Mg^{2+} ion has less preference for metal-EDTA complexes than the Ca^{2+} ion, the Mg was not dissolved into the extraction solution.

As previously reported (Wang et al., 2020; Yu et al., 2020, 2021; Wen et al., 2022), the degree and direction of the influence of MPs on the bioavailability of heavy

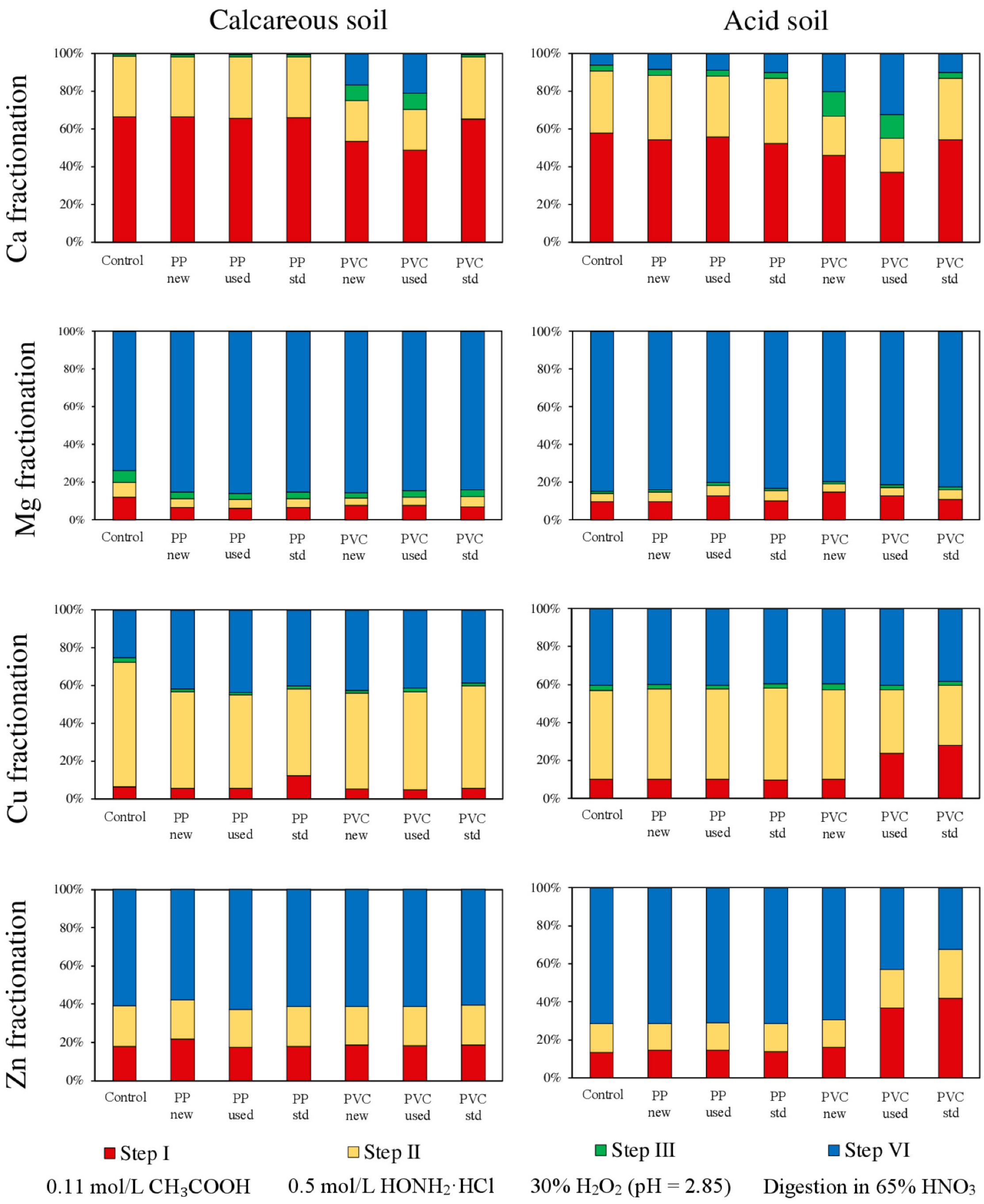


Fig. 4 Fractionation of macronutrients (Ca, Mg) and micronutrients (Cu and Zn) measured by four-step sequential extraction scheme (BCR-method) after 120 d of incubation for control (no MPs) and MPs polluted calcareous and acid soils. Grained PVC and PP ties were added to the soil: new ties purchased at viti-oenological local shop (PVC/PP new), previously used ties from the sampled vineyard (PVC/PP used) and polyvinyl chloride and polypropylene pure polymers (PVC/PP std).

metals in soil depends on the concentration and type of MPs, and a similar conclusion can be drawn for all nutrients in soil. Heavy metal bioavailability was positively correlated with microplastic size, soil sand concentration, and exposure time, but negatively correlated with soil pH and organic matter (An et al., 2023). Therefore, the binding mechanisms and mobility of nutrients in soil containing large abundance of micro-PVC need to be further investigated to clarify their bioavailability to plants.

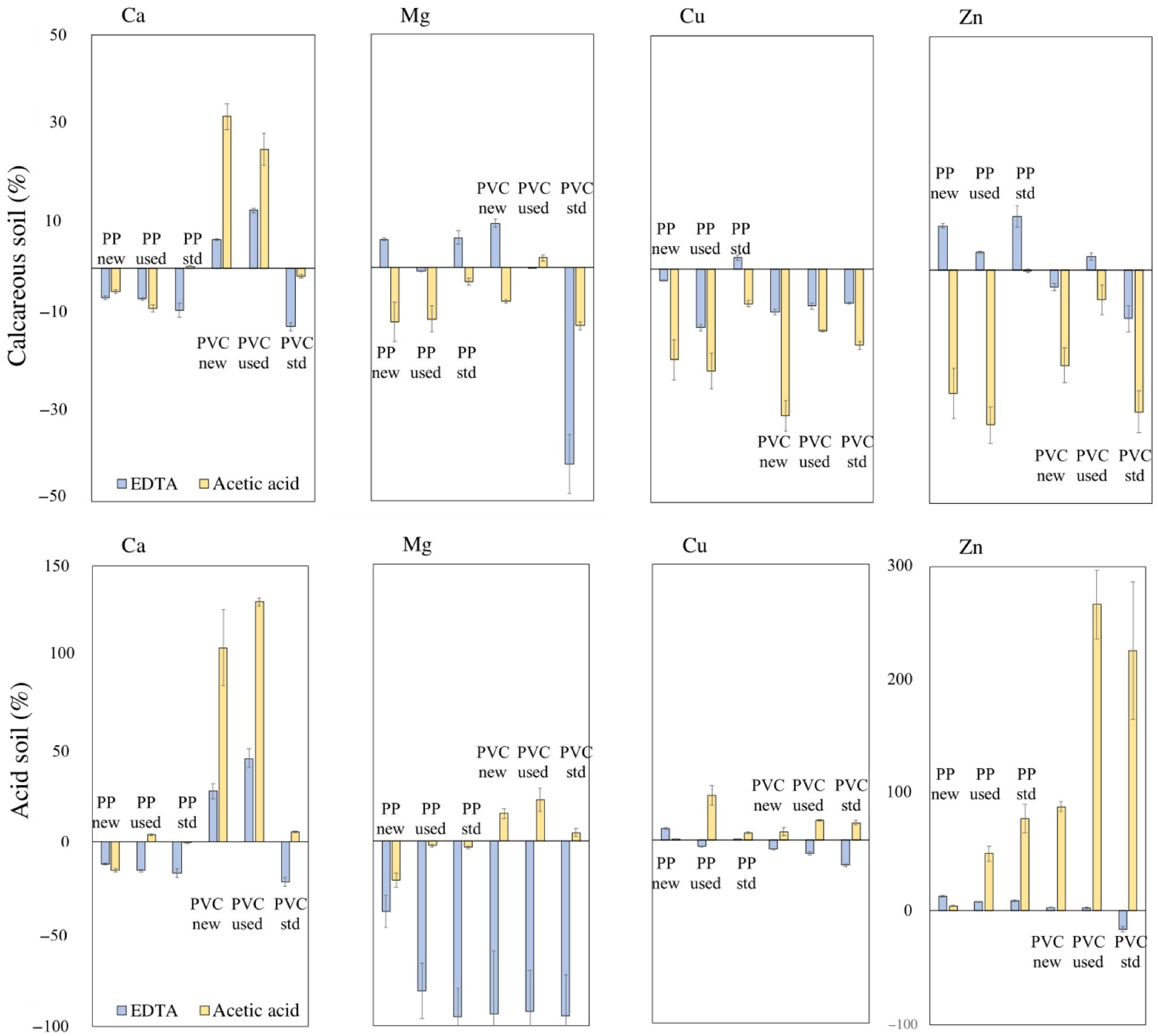


Fig. 5 Differences (expressed in percentage) in the bioavailability of macronutrients (Ca, Mg) and micronutrients (Cu and Zn) in MPs polluted calcareous and acid soils after 120 d of incubation, measured after EDTA (blue bars) and acetic acid (orange bars) single-step extractions. Grained PVC and PP ties were added to the soil: new ties purchased at viti-oenological local shop (PVC/PP new), previously used ties from the sampled vineyard (PVC/PP used) and polyvinyl chloride and polypropylene pure polymers (PVC/PP std). Positive values stand for increased bioavailability and negative values for reduced bioavailability of the plant nutrients in the soil. Error bars represent standard error of three replicates.

4 Conclusions

This study suggests that the type of plastic material (PP or PVC, plastic for commercial use or pure plastic polymers) plays an important role in determining impacts on important soil properties, including microbial activity and nutrient availability. Surprisingly, MPs derived from PVC exerted a more pronounced impact than those from PP, leading to significant alterations in the soil microbial community, as evidenced by increased soil basal respiration, increased microbial biomass, and enhanced microbial diversity. A particularly significant finding is the strong decrease in NO_3^- bioavailability that was induced by micro-PVC particles in both calcareous and acid soils. This pronounced negative effect, however, was only observed in soils treated with micro-PVC particles made from strings used in vineyards previously and not with the standard (i.e., pure) PVC material.

This study also demonstrated that soil type has a significant influence on the availability of nutrients in the presence of MPs particles. In acid soils, MPs significantly decreased the bioavailability of micronutrients (Fe, Cu, Mn, and Zn), and furthermore, MPs in acid soils also increased the pH and decreased the bioavailability of Mg and K. Conversely, calcareous soils are more resistant to the negative effect of MPs. Although a statistically significant increase in pH was also observed in calcareous soils, the presence of MPs had no influence on the availability of K, Ca, and Zn. The presence of micro-PVC particles altered the speciation of Ca: the acid-soluble fraction decreased, while Ca bound to soil organic matter and the crystalline lattice of primary and secondary minerals increased. In contrast, Zn in the acidic soil samples exhibited the opposite trend. For Cu, the association with Fe and Mn oxides decreased and the acid-soluble fraction increased.

From our results, we can conclude that high doses of PVC MPs in vineyard soils can negatively affect the availability of plant nutrients. The PVC plastic tube ties had a strong impact on soil microorganisms and the N cycle. Further studies are needed to clarify the role of additives and plasticisers, particularly on the microbial parameters.

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Conflict of Interests The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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