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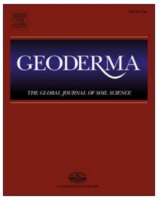

Climate warming masks the negative effect of microplastics on plant-soil health in a silt loam soil

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ABSTRACT

Many anthropogenic pressures are being exerted on terrestrial ecosystems globally, perhaps the most pressing of which include microplastics (MPs; <5 mm in size) pollution and climate change, both of which may have unpredictable consequences on soil ecosystem functioning. We therefore hypothesized that a dual pressure (MPs and warming) on plant-soil functioning would be more severe than either stress alone. Thus, we studied the interactive effects of MPs and warming on soil quality and ecosystem multifunctionality. Maize (*Zea mays* L.) was grown for 6 weeks under ambient and warming (+5 °C) conditions in the absence (control) or presence (5 % loading) of either polyethylene (PE), polyvinylchloride (PVC), or biodegradable polyhydroxyalkanoate (PHA). We found that PHA stimulated microbial biomass and enzyme activity due to the additional C resources, thus changing soil quality and ecosystem multifunctionality under ambient temperature. However, the accelerated microbial growth in PHA-treated soils also promoted N immobilization and plant–microbe nutrient competition, consequently decreasing plant health index by 65 % relative to the Control. As PVC and PE are chemically more stable than PHA, they had limited effect on soil quality and plant health under ambient temperature in the short term (6 weeks). Most of the negative impacts of MPs only occurred under ambient temperature, with few effects evident under warming conditions. This suggested that the effect of heat stress (evidenced by stunted growth and chlorophyll content) was noticeably more acute than the effect of MPs. In conclusion, we showed that MPs do affect plant health, soil quality, and ecosystem multifunctionality but these effects on plant-soil health were not exacerbated by the effects of a warmer climate.

1. Introduction

Many anthropogenic pressures are being exerted on terrestrial ecosystems globally, perhaps two of the most pressing being; microplastic (MP; < 5 mm in size) pollution and climate warming (Boots et al., 2019; Rillig, 2012), the consequences of which are still uncertain in the plant-soil system. Current estimates suggest that 359 million tons of plastic enter terrestrial ecosystems annually (Plastics Europe, 2020; Zhou et al., 2021b), with agro-plastics contributing ca. 6 million tons. Plastics may be introduced into the soil through a variety of pathways including sewage irrigation, fertilizer materials, waste disposal, atmospheric deposition (Mahon et al., 2017), and application of synthetic mulches (Sintim and Flury, 2017). Large plastic pieces may subsequently become fragmented via erosion, UV irradiation, high temperatures, tillage, and microbial decay (de Souza Machado et al., 2018b; Rillig, 2012), which in turn, leads to an accumulation of nano- and micro-sized plastics particles in soil (Nizzetto et al., 2016; Schwaferts et al., 2019; Zhou et al., 2022). With, an average of 571 and 263 particles kg^{-1} soil being reported in mulched and un-mulched Chinese farmlands, respectively (Büks and Kaupenjohann, 2020; Zhou et al., 2020a; Zhou et al., 2020b). This accumulation may adversely affect plant and soil health under conventional plastics pollution (Jiang et al., 2019; Qi et al., 2020; Zang et al., 2020b, 2022), but there is still uncertainty around the effect of biodegradable MPs on the agroecosystem. Given that the mean annual temperature is predicted to rise to 4.8 °C by the end of the twenty-first century (O'Neill et al., 2017), this additional stress adds further

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uncertainty to plant-soil responses to plastic pollution and loading. As a consequence, it is imperative to understand how climate warming and MPs affect agroecosystem functioning.

Soil provides a plethora of agroecosystem functions and services including carbon (C) sequestration and the provisioning of food resources, thus, understanding the impact of MPs in combination with climate warming is central to agricultural sustainability. MPs can cause indirect effects on plant health (Rillig et al., 2019a; 2021), for example by affecting the soil biophysical environment, i.e. decreasing soil bulk density, increasing soil aeration and evapotranspiration (de Souza Machado et al., 2018a; Wan et al., 2019), and altering soil pH (Boots et al., 2019; Qi et al., 2020). Additionally, MP addition may affect microbial activity and diversity (Jones et al., 2019; Zang et al., 2020b), which further affects enzyme production and nutrient cycling (Qi et al., 2018). However, the evidence is mixed, with MPs such as polyethylene (PE) and polyvinyl chloride (PVC) having been reported to suppress soil enzyme activities in some studies (Fei et al., 2020), but stimulate the activity of soil enzymes in others (Zang et al., 2020b; Zhou et al., 2021a). As a consequence, a consensus has not yet been reached on the impact of MPs on soil enzyme production and organic matter turnover. Given that soil enzymes are responsible for energy flow, and stimulation of C and nutrient cycling (nitrogen and phosphorus) (Trasar-Cepeda et al., 2008), the potential change in enzymes due to MPs pollution is likely to influence soil ecosystem processes. However, a single process cannot represent the complexity of agroecosystems in the real world. As such, it is still unclear how MPs impact on soil quality index (SQI; defined here as the sum of changes in soil biology, chemical and physical properties) and multiple ecosystem processes (i.e. ecosystem multifunctionality; EMF) simultaneously (Hector and Bagchi, 2007; Jia et al., 2022; Kuzyakov et al., 2020; Lozano et al., 2021).

Agroecosystems are also under increasing pressure from climate warming, with a projected increase in mean annual temperature of at least 4.8 °C by the end of the century (IPCC, 2019). This could potentially change soil and wider ecosystem processes and functions (Lozano and Rillig, 2020), and may adversely affect plant growth and health (Li et al., 2014). Warming is likely to increase the frequency and severity of extreme weather events (i.e. drought), exposing plants to longer growing seasons, exacerbating unpredictable physiological responses, and consequently affecting plant-soil health (Arshad et al., 2020). Increased plant productivity during warming may also induce nutrient imbalances and stimulate greater competition for nutrients between crops and microorganisms (Conant et al., 2011). As such, associated alterations in enzyme activity could then, in turn, alter C storage and nutrient cycling and have biogeochemical consequences (Sinsabaugh et al., 2009; Zang et al., 2020a). Given that global environmental change is a multifactorial phenomenon (Rillig and Lehmann, 2020), simultaneous interaction of warming and MPs pollution, is likely to give rise to large uncertainty in predicting effects (Kratina et al., 2019; Piggott et al., 2015; Xiang et al., 2021).

Therefore, we established a fully factorial mesocosm experiment growing maize (*Zea mays* L.) over 6 weeks, testing two factors; MP type (PE, PVC, and polyhydroxyalkanoate (PHA; a biopolymer)) and temperature (ambient and + 5 °C). PE and PVC were selected due to their frequent use as plastic mulching in the agroecosystem, while PHA represents a commercially available copolymer used for mulch film production (Dharmalingam et al., 2015; González et al., 2019; Zhou et al., 2022). Here, we aimed to: (1) illustrate the interactive influences of MPs and warming on plant health and soil quality; 2) evaluate the response of soil exoenzymes (involved in C, N, P cycling) and EMF to MPs under climate warming. We hypothesized that MPs would exacerbate the effects of thermal stress (i.e. warming), and this response would be greater in the presence of the bioplastic (PHA) in comparison to PE and PVC due to PHA-derived available C input.

2. Materials and methods

2.1. Soil sampling

A silty loam textured soil was collected from China Agricultural University's Experimental Station (N 37° 37′, E 116° 27′) in Wuqiao, Hebei province, China. The area has a monsoon-influenced continental climate, with a mean annual temperature of 12.9 °C and precipitation of 562 mm (Wang et al., 2020, 2022). The soil has a long-term history of maize production, without plastic mulching or any on-site record of plastic pollution. Additionally, the pre-experimental abundance of soil MPs was assessed following the methods of Grause et al. (2022) and Mausra and Foster (2015). The detailed method is given in supplementary information (Fig. S5). Approximately-six MP particles per 10 g soil were observed, representing a negligible fraction. Soil was air-dried after sieving (<5 mm), to remove the fine roots and other plant residues. The basic characteristics of the soil are shown in Table S1.

2.2. Experimental design

A pot experiment with a completely randomized design and five replicates (i.e. $n = 5$) was conducted in climate chambers (PQX-350H, Zhongyi Guoke Technology Co.) and set up at 25 °C (ambient: (day/night regime of 12 h/25 °C and 12 h/15 °C; relative humidity ~ 50 %, light intensity 40 %) and 30 °C (climate warming: day/night regime of 12 h/30 °C and 12 h/20 °C; relative humidity ~ 50 %, light intensity 40 %). These two temperatures represent the current average temperature during maize growth and a potential future climate warming scenario. Four treatments were included at each temperature; soil with no MP application (Control) and soil with the addition of polyethylene (PE), polyvinyl chloride (PVC), and poly (3-hydroxyalkanoates) (P3HB), respectively. P3HB is the most common form of PHA and the chemical basis of commercially available PHAs with a short methyl side chain and is a very crystalline and very brittle polymer. MPs powders modified from bigger commercial pellets were purchased from Zhonglian Plastics Technology Co., Ltd. (Fujian Province, China). MPs were manually mixed with 400 g soil (dry weight) to achieve a homogeneous concentration of 5 % (20 g, <120 μm diameter). An additional control treatment was included, with no plastic addition but with the equivalent amount of soil disturbance. This concentration of MPs was used to simulate a high level of MP pollution (e.g., in *peri*-urban environments) (Fuller and Gautam, 2016; Zang et al., 2020b). MPs < 1000 μm were used in this study as they were the dominant fraction in soil and sediment environments (Huang et al., 2021; Yu et al., 2021). Treated soil was then used to fill paper plant growth containers (172 mm height, 89 mm top diameter, 57 mm bottom diameter) without addition of any synthetic fertilizers (i.e., N, P, K) throughout the experiment. The Control treatment contained soil without MPs, but with comparable soil disturbance. Maize was selected since it is the second-largest food crop in China and contributes to one-third of national cereal production (FAO, 2012), and it is also one of the major crops grown in the North China Plain region. Three days after maize (*Zea mays* L. cv. Yongyu 2) germination on moist filter paper, four seeds were sown in each pot (later thinned to 1 seedling). The pots were irrigated using tap water every-two days and the soil moisture was maintained at 60 % water holding capacity (WHC) throughout the experiment by weighing. After each watering, the pots were randomly distributed in the respective chambers to minimize any location bias which may have existed. The experiment ran for 6 weeks in total.

2.3. Assessment of plant indices

Every-two days during incubation, plant height, number of leaves, primary and secondary leaf length, leaf width, and leaf area were measured and recorded. Further, the leaf chlorophyll content (SPAD value) was analyzed using a portable chlorophyll meter (SPAD-502 Plus, Konica Minolta, Tokyo, Japan) from 10:00 am to 12:00 pm. Three leaves of each plant were measured for SPAD (Yang et al., 2014).

After 42 days of growth, the maize plants were destructively harvested and separated into shoot and root for further analysis. Roots were removed from the soil and washed gently. Subsequently, roots were placed in a clear Perspex tray with a film of saline solution and scanned with a modified flatbed scanner (EPSON Perfection V800, Seiko Epson, Nagano, Japan). WinRHIZO software (Regent Instruments, Quebec City, Canada) was used to measure root length and volume, average root surface area and root diameter, total root surface area. Plant biomass (i. e. shoot and root) were determined after oven drying at 80 °C until the samples reached a constant weight. It should be noted that three plants in Control and two plants in PE treatment died during the experiment under the higher temperature regime (30 °C). The five dead plant and soil samples were excluded from further analysis.

2.4. Soil biochemical analysis

The soil pH was determined using a glass electrode meter (FiveEasy Plus FE28, Mettler Toledo, China) in a soil and distilled water suspension (1: 2.5, w/v). Electrical conductivity (EC) was measured using an EC meter (DDS-11A, INESA, China). Soil organic carbon (SOC) was determined using the potassium dichromate ($K_2Cr_2O_7$) oxidation–reduction titration method (Bao, 2000; Yan et al., 2022). Available phosphorus (Avail P) was extracted from 5 g soil suspended in 20 ml 0.5 M sodium bicarbonate ($NaHCO_3$), filtered through a Millipore 0.45-µm filter, and measured using a microplate spectrophotometer using the molybdate blue method of Murphy and Riley (1962). Microbial biomass carbon (MBC) and nitrogen (MBN) were calculated following the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, the soil was carefully mixed and 5 g of soil were directly extracted using potassium sulfate (K_2SO_4) (20 ml, 0.05 M). A further 5 g of soils were fumigated with chloroform for 24 h and then extracted in the same manner. The extracts were analyzed for their total C and N concentration using a 2100 TOC analyzer (TOC-L CPN, Shimadzu, Japan). The unfumigated samples were used to measure NH_4^+, NO_3^-, dissolved organic C (DOC), and total dissolved N (TDN). The total amount of MBC and MBN were calculated based on the difference of K_2SO_4-extracable C and N between fumigated and non-fumigated samples using the K_{EC} and K_{EN} factors as 0.45 and 0.54, respectively.

2.5. Soil exoenzyme activities

The activities of six exoenzymes, involved in C acquisition (β-cellobiohydrolase (CE, EC 3.2.1.91), β-xylosidase (BX, EC 3.2.2.27) and β-glucosidase (BG, EC 2.2.1.21)), N acquisition (β-1,4-*N*-acet-ylglucosaminidase (NAG, EC 3.2.1.52) and L-leucine aminopeptidase (LAP, EC 3.4.11.1)), as well as phosphorus (P) acquisition (acid phosphatase (AP, EC 3.1.3.2)) were determined (Sinsabaugh and Shah, 2012; Zhou et al., 2020b). Two synthetic fluorescence indicating substrates; 7-amino-4-methylcoumarin (AMC) and 4-methylumbelliferone (MUF), were used to analyze the potential activities of exoenzymes. Briefly, a homogenized solution of 1 g fresh soil and 50 ml sterile water were shaken for 30 mins. 50 µL aliquots from the slurry were then pipetted into 96 well-white microplates. Afterward, 50 µL buffer (0.1 M MES buffer or 0.05 M Trizma® buffer), 100 µL corresponding substrate at a concentration of 200 µM were added to the microplates. Enzyme activities (nmol $g^{-1}h^{-1}$) were determined by measuring the microplates fluorometrically (excitation wavelength 360 nm, emission wavelength 450 nm) at 0, 30, 60, and 120 min with a microplate reader (Multiskan Go 1510, Thermo Scientific, Waltham, Massachusetts, USA).

2.6. Calculations

2.6.1. Soil quality and plant health index

Soil quality index (SQI) was evaluated by converting each biotic and abiotic soil factor to a value of 0–1 using the following equations:

$$\mathrm{SL}_i = \frac{X}{X_{\max}} \tag{1}$$

$$\mathrm{SL}_i = \frac{X_{\min}}{X} \tag{2}$$

where “$\mathrm{SL_i}$” is the linear score of the factor “i” varying from 0 to 1, “X” denotes the measured value, “X_{max}” and “X_{min}” are the maximum and minimum mean value of the factor “i”, respectively. Based on the sensitivity of soil quality, soil parameters were divided into two functions. A “more is better” scoring curve was used for each parameter where increases results in an improvement in soil quality, (e.g., SOC, MBN, MBC), otherwise a “less is better” scoring curve was used (e.g., pH).

SQI was calculated using an SQI-area approach by comparing the area on a radar graph comprising of all soil parameters (Kuzyakov et al., 2020):

$$\mathrm{SQI\ \text{-}\ area} \ = \ 0.5 \cdot \sum_{i}^{n} SL_i^2 \cdot \sin\left(\frac{2\pi}{n}\right) \tag{3}$$

where *n* is the number of parameters or factors used for the SQI-area.

Similarly, plant health index (PHI) was determined according to (Eq. (1)) using a linear normalization as proposed for the soil quality index (SQI) following Andrews et al. (2002) and Zeraatpisheh et al. (2020).

A “more is better” scoring curve was used as the level of each parameter increased with the improvement in plant health. PHI was assessed using a PHI-area approach by comparing the area on a radar diagram made with all plant parameters same as SQI using (Eq. (3)) (Kuzyakov et al., 2020; Ma et al., 2022).

2.6.2. Microbial metabolic limitation and ecosystem multifunctionality

Enzyme activities belonging to the same functional group were normalized to evaluate the activities of enzymes involved in C (BG, BX, CE), N (NAG and LAP) and P (AP) cycling (Jia et al., 2022; Luo et al., 2018; Xu et al., 2022). For example, C acquisition was calculated using the followings:

$$\mathrm{C-acq} \ = \ \frac{BG + BX + CE}{3} \tag{4}$$

The microbial metabolic limitation was determined by calculating the vector lengths and angles of enzymatic activity based on untransformed proportional activities:

$$\mathrm{x} = [(BG + BX + CE) \ / \ (BG + BX + CE + AP)] \tag{5}$$

$$\mathrm{y} = [(BG + BX + CE) \ / \ (BG + BX + CE + LAP + NAG)] \tag{6}$$

Vector lengths were calculated as the square root of the sum of x^2 and y^2 (Eq. (7)). The relative activity of C-versus P acquiring enzymes and C-versus *N*-acquiring enzymes were represented by × and y, respectively (Moorhead et al., 2016). Vector angles were calculated as the arctangent of the line extending from the plot origin to point (x, y) (Eq. (7)).

$$Vector\ lenght = SQRT\left(x^2 + y^2\right) \tag{7}$$

$$Vector\ angle(^\circ) = DEGREES(ATAN2(x + y)) \tag{8}$$

Normally, the vector angles higher than 45° represent P limitation, whereas lower than 45° represent N limitation. The longer vector lengths indicate stronger microbial C limitation, while higher vector angle indicate stronger microbial P limitation (Jia et al., 2022; Ma et al., 2022).

Ecosystem multifunctionality (EMF) was evaluated using activities of six exoenzymes (BG, CE, BX, NAG, LAP and AP). Enzyme activities were standardized using Z-score (Eq. 9), later averaged to obtain multifunctionality index (Delgado-Baquerizo et al., 2016).

Z-score = (x-mean$_i$) / SD$_i$ (9).

where “x” is the measured enzyme activity; “mean” is the average activity of enzyme “i” and “SD” is the standard deviation of the enzyme “i”.

2.7. Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics software version 25.0 (IBM Corp., Armonk, NY). The values presented in the figures are means ± standard errors (SE). Data were first tested for normality using the Shapiro-Wilk test ($p < 0.05$) and homogeneity of variance using the Levene test $p < 0.05$. Significant differences between the temperature (temp) and microplastic (MPs) treatments and their interaction (temp × MPs) were analyzed using a two-way ANOVA (analysis of variance) in combination with Fishers Least Significant Difference (LSD) test. If temperature (T_1, T_2) was significant according to two-way ANOVA, then One-way ANOVA was performed to show the significant difference between MPs treatments at each temperature. Radar graphs of the relative responses of plant and soil properties to temperature and MPs as well as bar graphs representing the comparative analysis of soil indexes and enzyme activities were visualized in Sigmaplot version 12.5 (Systat Software Inc., San Jose, CA). Pearson correlations between plant and soil biochemical, as well as enzymes factors at 25 and 30 °C were visualized in R (Version 4.0.3) using package “corrplot”. A Random Forest analysis was conducted to identify the main predictors of PHI among soil biotic and abiotic factors using the “randomForest” package in R (Version 4.0.3). The significance values of the cross-validated R^2 and the whole model were examined using “3” function in “A3” package in R. Moreover, the significance of each predictor on the response variables was assessed using the “rfPermut” function in R “rfPermute” package (Chen et al., 2018). Heat maps were analyzed using “circlize” package in R (Version 4.0.3) and visualized “ggplot2” package in R (Version 3.5.0) software.

3. Results

3.1. Plant growth and health

Plant growth was significantly affected by MPs and temperature (Fig. 1). MPs presence decreased plant height and SPAD at both temperatures compared to Control ($p < 0.05$, Fig. 1a), while the MPs effect was stronger at ambient than warming (Fig. 1). Root biomass was decreased by PHA (54 %) and PVC (5.9 %) addition under ambient. In contrast, PE addition increased root biomass by 23 % under ambient conditions, but decreased by 8–38 % under warming ($p < 0.05$). Average root length, root volume, and total root surface area were decreased by PHA addition in both ambient and warming conditions ($p < 0.05$). PE addition increased root volume and total root surface area by 57 % and 21 % at ambient, whereas it decreased root volume and total surface area by 21 % and 43 % under warming, respectively ($p < 0.05$). At ambient temperature, PHI was decreased by 65 % and 18 % with the addition of PHA and PVC, respectively, whereas it was increased by 30 % under PE ($p < 0.05$; Fig. 1 c). However, there was no significant difference in PHI between MPs and the Control treatment under warming ($p > 0.05$, Fig. 1 c).

3.2. Soil biochemical properties and quality index

The addition of PHA decreased N_{min} by 90–98 % at both temperatures in comparison to the Control ($p < 0.001$; Fig. 1). PVC addition decreased N_{min} by 13 % but increased TDN by 20 % under warming. PHA and PE addition caused a reduction in TDN at both temperatures ($p < 0.05$), whilst this MPs effect was stronger under warming than under ambient temperature ($p < 0.001$). Available P was decreased by PHA

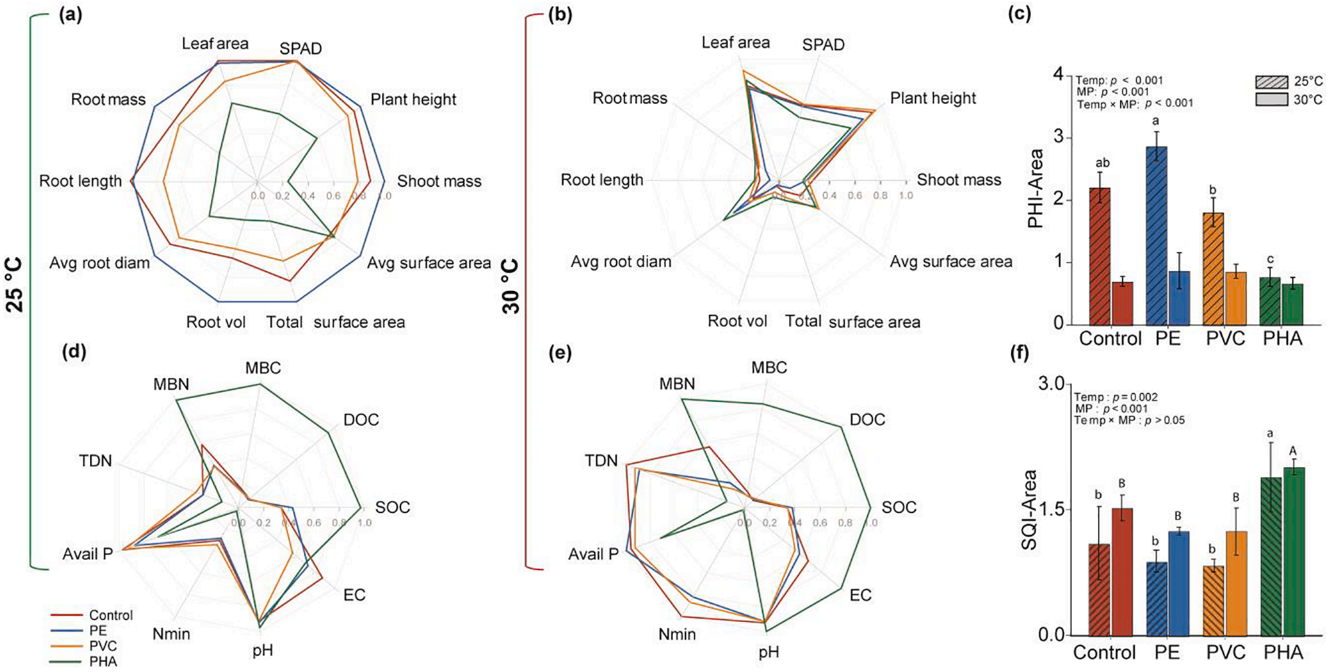

Fig. 1. Radar charts relative response of plant and soil parameters at 25 °C (a, d) and 30 °C (b, e), plant health index (PHI) (c) and soil quality index (SQI) (f) as affected by Polyethylene (PE), Polyvinyl chloride (PVC), and Polyhydroxyalkanoates (PHA) microplastics. Results are expressed on a mean basis ± SEM ($n = 5$). Lower- and upper-case letters represent significant differences based on one-way ANOVA followed by LSD test at 25 °C and 30 °C, respectively. p value for temperature (Temp), microplastics (MP) and their interaction (Temp × MP) was based on a Two-way ANOVA. Plant factors including leaf area, soil plant analysis development (SPAD), plant height, shoot dry mass (Shoot mass), root dry mass (Root mass), root length, average root diameter (Avg diam), root volume (Root vol), average root surface area (Avg surface area), total root surface area (Total surface area). Soil factors including: microbial biomass carbon (MBC), dissolved organic carbon (DOC), soil organic carbon (SOC), electrical conductivity (EC), pH, soil mineral nitrogen (N_{min}), available phosphorus (Avail P), total dissolved nitrogen (TDN), and microbial biomass nitrogen (MBN).

and PVC ($p < 0.05$), however it did not change in response to PE at both temperatures. Furthermore, SOC and MBN increased by PHA compared to the Control regardless of the temperature ($p < 0.001$; Fig. 1d, e). The DOC concentration was also 7.5 and 10.5 times higher in PHA-treated soil respectively under ambient and warming conditions than that in the Control ($p < 0.05$).

In addition, PHA addition increased SQI by 72 % at ambient and 32 % at warming, respectively. By contrast, PVC and PE addition did not alter SQI at both temperatures ($p > 0.05$; Fig. 1f).

3.3. Soil exoenzyme activities and stoichiometry evaluation

All enzymes responded differently to temperature and MPs addition (Fig. 2a, b, c). Compared to the Control, C-acquisition enzyme activity was 70 % higher in PHA-treated soil whereas it was 19 % and 8.2 % lower in PVC and PE-treated soil at ambient temperature ($p < 0.05$; Fig. 2a). In contrast, PHA caused 3.5 times and 1.4 times higher in N-acquisition enzymes under corresponding ambient and warming as compared to the Control. However, PE and PVC did not change N-acquisition enzyme activity at either temperature ($p > 0.05$; Fig. 2b). In addition, P-acquisition (AP) was 39.6 % and 21.6 % higher in PHA-treated soil respectively at 25 °C and 30 °C, while PE (9–13 %) and PVC (12 %) decreased P-acquisition enzymes in comparison to control at both temperatures ($p < 0.05$; Fig. 2c).

Although the characteristics of the enzyme stoichiometries responded similarly to temperature, it was changed by MPs addition (Fig. 3). All sample points were lower than the line of slope 1 (Vector angle < 45°), indicating that the microbial community was limited by N, especially in the presence of PHA (Fig. 3a). Vector length and angle were decreased by PHA in comparison to Control soil at both temperatures ($p < 0.05$). Although PVC and PE did not change vector length at both temperatures as compared to the Control ($p > 0.05$), they had a lower vector angle under warming (Fig. 3c). In addition, there was a significant positive correlation between microbial C limitation (vector length) and microbial N limitation (vector angle) at both temperatures ($p < 0.001$; Fig. 3d).

3.4. Ecosystem multifunctionality

MPs significantly affected the EMF and the EMF:MBC ratio at both temperatures ($p < 0.001$; Fig. 2d, Fig. S1). PHA addition resulted in a 7 % higher EMF at ambient and 15 % higher at the higher temperature. In contrast, EMF was decreased by PVC and PE addition at both temperatures, compared to the Control ($p < 0.05$; Fig. 2 d). Compared to ambient conditions, EMF in soil with PVC and PE was higher under the warming treatment. Additionally, the EMF: MBC ratio was increased by PHA addition while PE and PVC lowered the ratio ($p < 0.05$; Fig. S1b).

3.5. The relationships between soil biotic, abiotic factors, and PHI

PHI was negatively correlated with SQI and EMF at ambient temperature ($p < 0.01$; Fig. 4b), whilst a positive correlation between EMF and SQI was found at both temperatures (Fig. 4c). Specifically, SOC, MBC, MBN, and DOC showed a strong negative correlation with PHI, while available P, N_{min} were positively correlated with PHI under ambient temperature conditions. Similarly, plant factors and CE enzyme were positively correlated with PHI whereas other enzymes (BG, LAP, AP BX) showed a strong negative correlation with PHI under ambient conditions (Fig. 5a). In addition, plant factors such as PH, SM, LA, and SPAD possessed a positive correlation with PHI under warming. The contributions of soil biotic, abiotic factors attributing to PHI were estimated using the Random Forest Model ($p < 0.005$; Fig. 5 b), which explained 56 % of the variation in PHI, with TDN, N_{min}, DOC, AP, and MBC being identified as the main driving factors.

4. Discussion

4.1. Plant growth and health

Our results documented that the effects of MPs on plant growth and health were greatly dependent on plastic type (Lehmann et al., 2019; Rillig et al., 2019a; Zhou et al., 2021b). We found that the addition of PE, PVC and PHA lowered the seed germination rate (Fig. S4). This was in

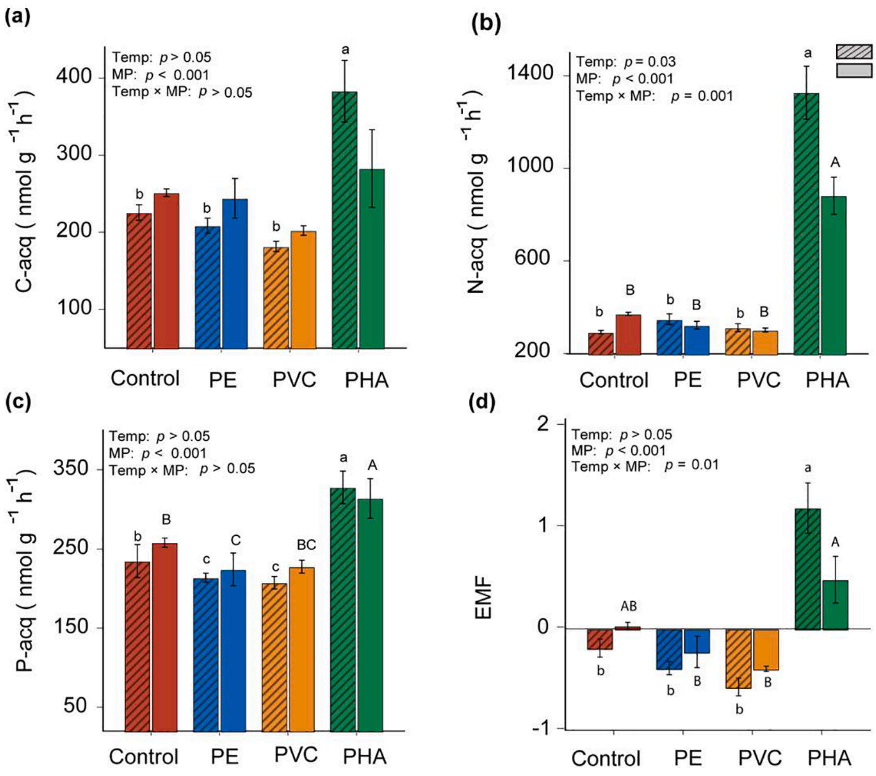

Fig. 2. Carbon acquiring enzymes (C-acq, a), nitrogen acquiring enzymes (*N*-acq, b), and phosphorus-acquiring enzymes (P-acq, c), as well as ecosystem multifunctionality (EMF, d) in soil without (Control), and with Polyethylene (PE), Polyvinyl chloride (PVC), and Polyhydroxyalkanoates (PHA) microplastic at 25 °C and 30 °C. Results are expressed on a mean basis ± SEM ($n = 5$). The lower- and upper-case letters show significant differences based on one-way ANOVA and followed by LSD test, and p value for temperature (Temp), microplastics (MP), and their interaction (Temp × MP) based on a two-way ANOVA. C-acq: β-1,4-glucosidase, β-cellobiosidase, and β-cellobiohydrolase; *N*-acq: β-1,4-*N*-acetylglucosaminidase and ʟ-leucine aminopeptidase; P-acq: acid phosphatase. The temperature legend is the same for all panels.

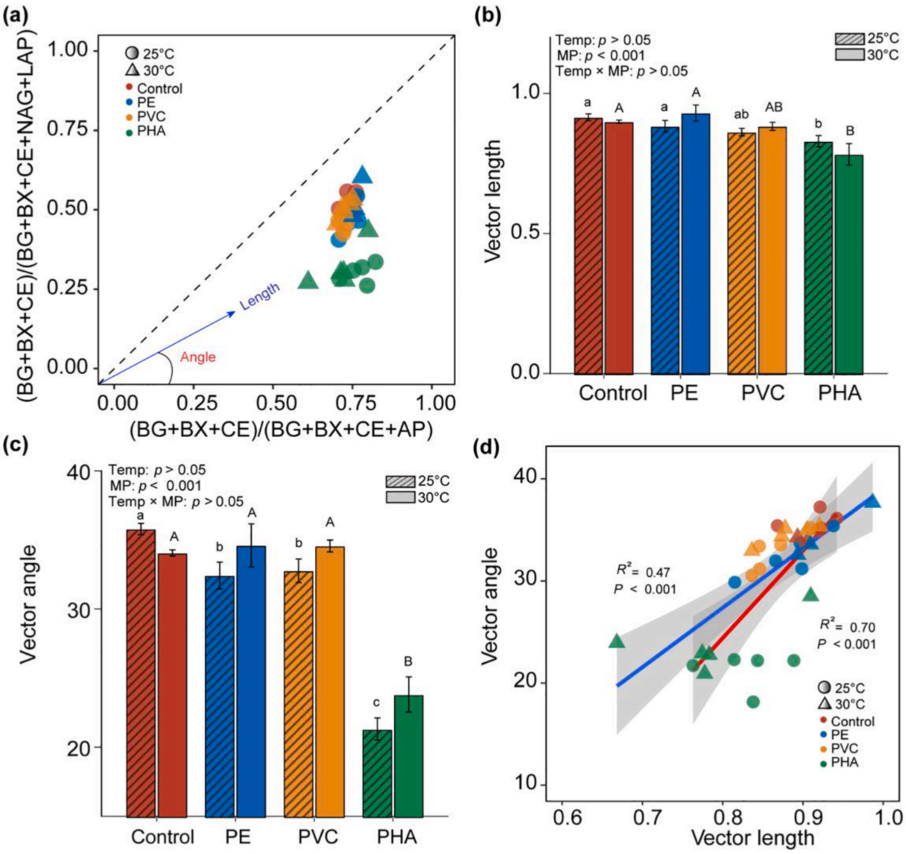

Fig. 3. Extracellular enzyme stoichiometry of the relative proportions of C to N acquisition versus C to P acquisition (a), the variation of vector length and angle (b and c), and their relationships (d). BG: β-glucosidase; BX: β-xylosidase; CE: β-cellobiohydrolase; NAG: β-1,4-N acetylglucosaminidase; LAP: L-leucine aminopeptidase; AP: acid phosphatase. Results are expressed on a mean basis ± SEM ($n = 5$). The lower- and upper-case letters show significant differences based on one-way ANOVA and followed by LSD test, and p value for temperature (Temp), microplastics (MP), and their interaction (Temp × MP) based on a two-way ANOVA. Linear regression analysis was used to identify the relationships of microbial C limitation with microbial N/P limitation. Solid lines indicate the model fits between vector angle and vector length. Grey areas show the 95 % confidence intervals of these models.

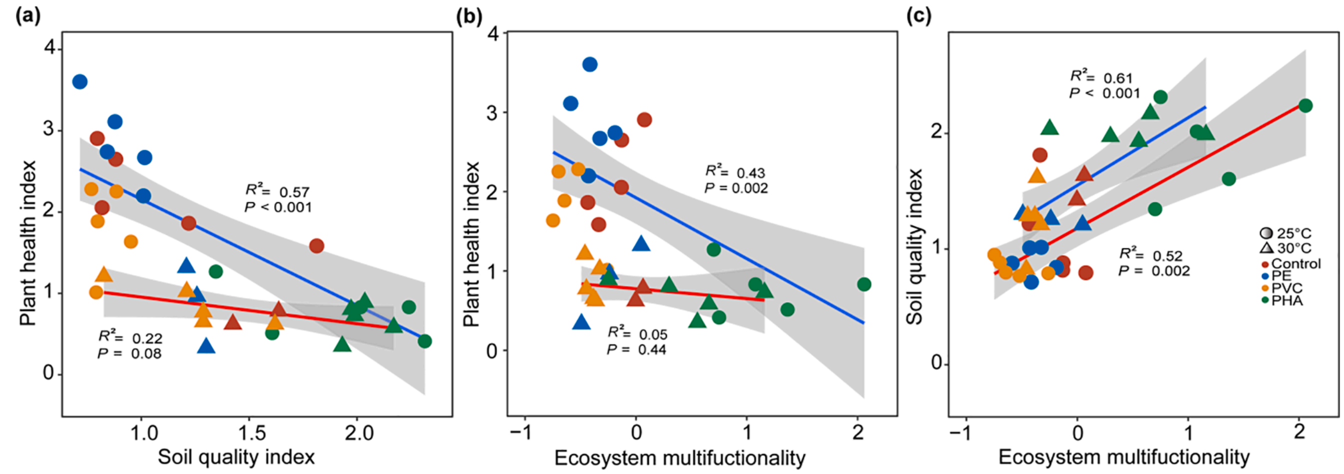

Fig. 4. Scatter plot describing the correlation between soil quality index (SQI) and plant health index (PHI) (a), PHI and ecosystem multifunctionality (EMF) (b), as well as SQI and EMF (c), as affected by Polyethylene (PE), Polyvinyl chloride (PVC), and Polyhydroxyalkanoates (PHA) microplastics at 25 °C and 30 °C. Solid lines indicate the model fits among the SQI, PHI, and EMF. Grey areas show the 95 % confidence intervals of these model fits. The legend is the same for all panels.

line with previous studies reporting up to 67 % decline in seed germination caused by MPs (conventional; bioplastics) (Bosker et al., 2019; De Silva et al., 2021). Under ambient temperature, PVC decreased plant health, while PE increased plant health (Fig. 1c). This was consistent with Pignattelli et al. (2020) who found that PVC induced oxidative stress and exerted the highest photo-toxicity in *Lepidium sativum* L. when grown in the presence of either PE, PVC, or polypropylene (PP), likely due to the impurities and additives in the polymers (de Souza Machado et al., 2019). For example, chloride contained in the PVC may have leached out and consequently caused stronger toxicity to the plant compared with PE (Fuller and Gautam, 2016). In contrast, however, the presence of PE in soil has been reported to enhance root growth by enhancing soil aeration (de Souza Machado et al., 2018a; Wen et al., 2020), which has the potential to allow better root exploration of the soil matrix. This was supported in our study where higher plant height, leaf area, as well as root biomass was observed in the PE- relative to PVC-treated soils (Fig. 1a). While the increasing trend was observed for plant biomass and root traits (i.e. root diameter, root volume), however, this was not the case for leaf chlorophyll content (i.e., SPAD measurements in our case) in the absence of thermal stress (i.e. raised temperature). This indicates that PE did not greatly affect photosynthesis and supported an increased C flow from above- to below-ground biomass.

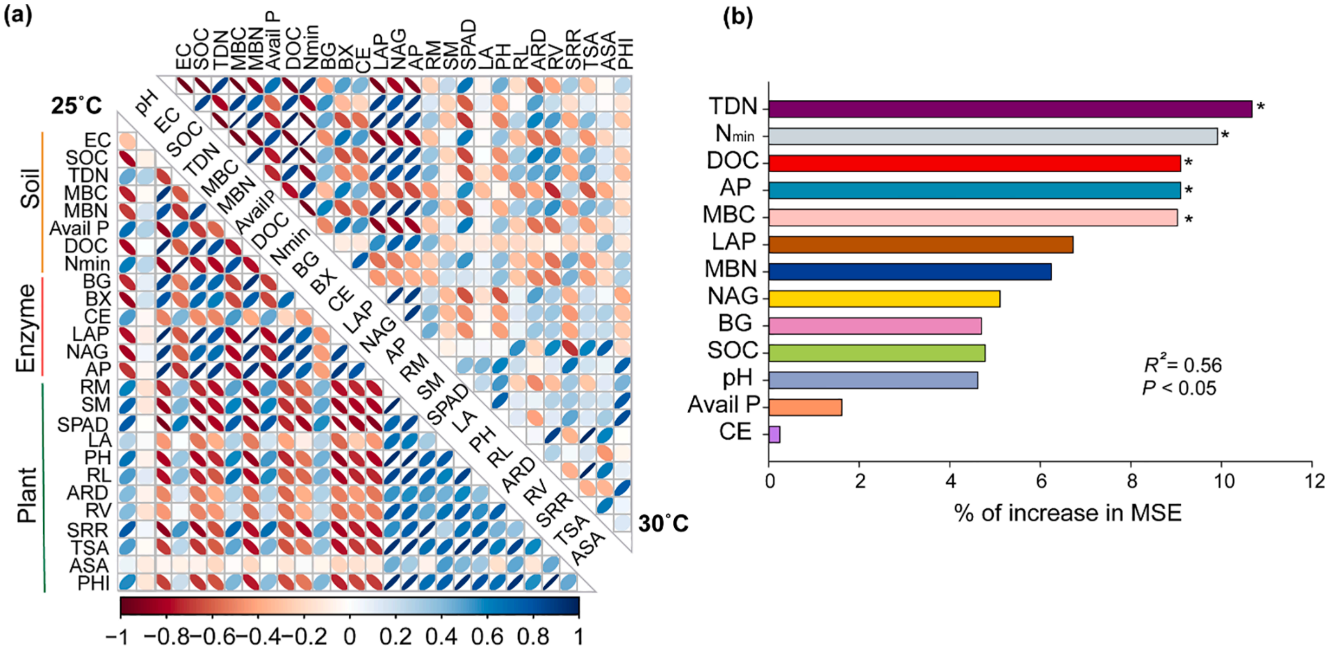

Fig. 5. Correlation between soil, plant factors, and enzyme activity at ambient and warming temperature (a). Soil factors including: pH, electrical conductivity (EC), soil organic carbon (SOC), total dissolved nitrogen (TDN), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), available phosphorus (avail P), dissolved organic carbon (DOC), soil mineral nitrogen (N_{min}). Plant factors include: root dry mass (RM), shoot dry mass (SM), soil plant analysis development (SPAD), leaf area (LA), plant height (PH), root length (RL), average root diameter (ARD), root volume (RV), shoot root weight ratio (SSR), total root surface area (TSA), average root surface area (ASA) and plant health index (PHI). Enzyme activities include: β-glucosidase (BG), β-xylosidase (BX), β-cellobiohydrolase (CE), β-1,4-N acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP) and acid phosphatase (AP). Random Forest mean predictor importance (% of increase of MSE) of soil and plant parameters on PHI. Significance levels of each predictor are as follows: * $p < 0.05$.

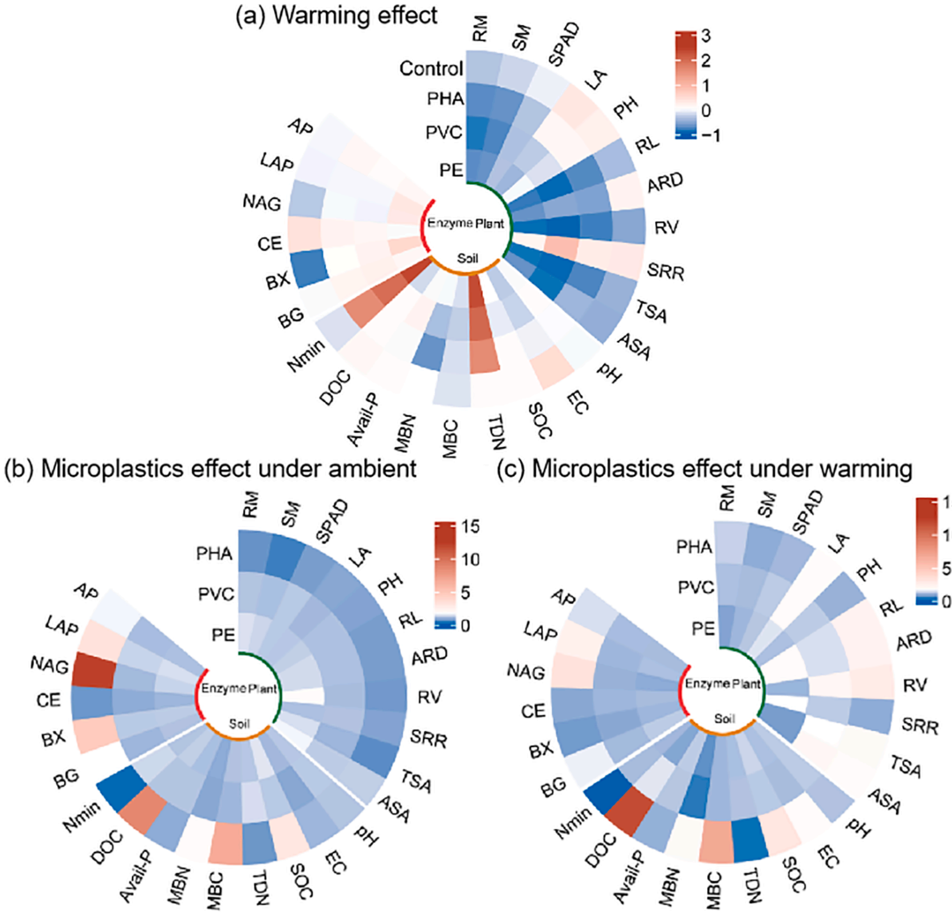

Fig. 6. Relative warming effect (%) in the control and microplastics treatments (PE, PVC, PHA) in response to warming (30 °C) compared to ambient conditions (25 °C) on soil factors, plant factors, and soil exoenzyme activities (a), and the effect of microplastic (PE, PVC, PHA) addition on these factors at 25 °C (b), and 30 °C (c). The difference of soil, plant and enzyme activities were normalized and converted to a color scale; an increase and decrease in activity being indicated by the intensity of red and blue color. Plant: root dry mass (RM), shoot dry mass (SM), soil–plant analysis development (SPAD), leaf area (LA), plant height (PH), root length (RL), average root diameter (ARD), root volume (RV), shoot root weight ratio (SRR), total root surface area (TSA), average root surface area (ASA); soil: pH, electrical conductivity (EC), soil organic carbon (SOC), total dissolved nitrogen (TDN), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), available phosphorus (avail P), dissolved organic carbon (DOC), soil mineral nitrogen (N_{min}); enzyme: β-glucosidase (BG), β-xylosidase (BX), β-cellobiohydrolase (CE), β-1,4-N acetylglucosaminidase (NAG), L-leucine aminopeptidase (LAP) and acid phosphatase (AP).

Chlorophyll content and ROS (reactive oxygen species) production for oxidative stress may need to be evaluated to better understand the effect of different types of MPs on plant performance. Unlike traditional chemically stable polymers (i.e., PE and PVC), the presence of biodegradable PHA at ambient temperature resulted in severe adverse effects on seed germination, plant performance and the overall PHI score (Fig. 1c). Specifically, it appeared to promote nutrient immobilization in the soil (e.g., N), and thus resulted in greater plant–microbe competition for nutrient resources (Fig. 1c). Thus, in this instance biodegradable MPs were more toxic (i.e. reduced plant performance and health), compared with conventional MPs.

Warming had a more profound impact on plant performance than MPs (Fig. 6; Fig. S2). In other words, thermal stress masked the effect of MPs on plant health. Warming severely constrained plant growth, chlorophyll formation, and presumably increased water demand and loss, ultimately resulting in inefficient photosynthesis and stunting (Lobell et al., 2013). Moreover, plant shoot–root signaling response to heat stress also resulted in further alteration in root architecture and growth suppression (Lamaoui et al., 2018; Lipiec et al., 2013). This was supported by a significant decrease in a range of below-ground parameters due to warming (Fig. S2). Our results are consistent with the optimal temperature for maize growth ranging from 25 to 33 °C during the day, but importantly from only 17 to 23 °C at night (Waqas et al., 2021). However, the temperature of 30 °C would be classed as mild thermal stress with the most direct negative effects on plant metabolism occurring at temperatures exceeding 35 °C (Hagedorn et al., 2019; Neiff et al., 2016).

As well as having a direct effect on plant growth, higher temperatures may also indirectly (via rhizodeposition) or directly alter microbial activity in soil (D`Alò et al., 2021; Geisseler et al., 2011). Warming can stimulate microbial activity and growth, which in turn, may increase the degradation of MPs. As a consequence, more toxic additives inside the MPs may be released into the soils and be uptaken by plants. Subsequently, the MPs effect on plant health was similar between different types of MPs under warming. This was also supported by the finding that rising temperature increased the toxicity of MPs to *Pomatoschistus microps* (Fonte et al., 2016). Collectively, our results documented that the impact of MPs on plant performance and health is altered when climate warming is considered.

4.2. Soil biochemical properties and soil quality index

In the present study, no significant shifts in soil biochemical properties and soil quality index were observed after being exposed to PVC and PE under ambient conditions (Fig. 1f). This was attributed to the resistant hydrocarbon structure of traditional polymers (de Souza Machado et al., 2019). Furthermore, our current experiment lasted 42 d, and such a short exposure might limit the release of additives from PE and PVC under ambient temperature. Comparatively, biodegradable MPs are more readily decomposed by soil microbes and act as a source of soluble organic C (Rillig et al., 2021; Zhou et al., 2021a). Moreover, the higher soluble C content in PHA-treated soil increase soil C:N ratio, and subsequently led to soil N immobilization (Rillig et al., 2019b; Qi et al., 2020). Another report by Zhou et al. (2021a), also concluded that C from biodegradable plastic can stimulate microbial growth and biomass turnover. Consequently, higher microbial biomass in PHA-treated soil led to higher soil quality as compared to PVC and PE.

Rising soil temperature may lead to more rapid fungal growth to promote MPs degradation and thus the release of potentially toxic additives (Hahladakis et al., 2017; Liang et al., 2019). However, warming did not alter soil properties compared with ambient temperature, and the soil quality was similar between control and traditional polymers (PE and PVC) under warming (Fig. 1f). We ascribe this to the innate ability of soils to withstand a range of abiotic stresses (Lee et al., 2017). This buffering capacity of soil further explained the unchanged SQI whilst the plant health index (PHI) changed under soil contaminated with MPs, since plants are more sensitive to environmental stress.

4.3. Soil exoenzyme activities and stoichiometric ratio

Under ambient temperature, PHA increased all the assessed enzyme activities (Fig. 2a), which was ascribed to the increased soluble C with PHA addition, as discussed above. PHA serves as a source of C and energy for microbial growth and subsequently increases microbial activity (Zhang et al., 2020). Accordingly, higher metabolizable C increased soil MBC (Fig. 1a) and accelerates the utilization and assimilation of available nutrients in order to maintain their stoichiometric ratio (Jia et al., 2022), thus induced microbial N limitation. This was in line with the lower content of N_{min} (Fig. 1a) and lower vector angle in PHA-treated soils compared with other treatments (Fig. 3). Although the addition of PE and PVC in soil serves as a C source, the chemical bonds within conventional MPs are relatively stable, making it difficult for bacteria and fungi to utilize the C resource, resulting in few direct impacts on microbes and their associated enzymes (Brown et al., 2021; Singh and Sharma, 2008). In addition, MP breakdown is unlikely to occur during a short-term incubation such as that undertaken here and in previous studies (Liu et al., 2022; Pham et al., 2021; Song et al., 2017). Therefore, soil enzyme activity did not change under PE and PVC contamination.

Our results revealed that biodegradable MPs contamination in soil under warming inhibits the activities of C- and N-acquiring enzymes (Fig. 2a, b). This may be ascribed to the stronger toxic effects on microbes due to the larger amounts of additives released from MPs caused by warming. We also speculate that the substrate limitation by MPs adsorption was intensified under warming, which resulted in inhibited soil enzyme activities. Given that the stimulated microbial growth and enhanced enzyme production are responsible for the breakdown of biodegradable MPs under warming, it is also likely to increase the microbial C and N limitation in MPs polluted soils. This was supported by the higher vector length and angle in warmed than in ambient soils (Fig. 3b, c). However, such differences between ambient and warming are also explained by the fact that different MPs (and their associated chemical additives, some of which might be toxic; Rillig et al., 2021), but it is not clear how each of these factors contributed to the effects observed here.

4.4. Effects of MPs and warming on EMF

Due to its stable polymer backbone structure, PE addition led to no significant change of soil EMF under warming (Fig. 2d). However, additives like bisphenol A contained in PVC polymers may have leached into the soil and caused stronger toxicity to microbes (Suhrhoff and Scholz-Böttcher, 2016; Tarafdar et al., 2022), which then led to reduced C-acquiring enzyme production (Fig. 2a). Therefore, PVC induced a stronger inhibition on EMF than PE (Fig. 2d). Since biodegradable MPs like PHA might provide optimal microbial growth niches and enhanced enzyme production (Zhou et al., 2021a), it is assumed that PHA offsets some negative effects of physical disturbance and its indirect impact on the microbiome, thus increasing EMF (Fig. 2d). In addition, biodegradable MPs can also increase soil water evaporation (Wan et al., 2019), improve soil aeration and porosity, thus altering the soil redox state and the subsequent chemical form of elements. Some of these elements (e.g., iron and manganese) are cofactors of enzymes involved in biochemical reactions (Tebo et al., 2004). The improved bioavailability of these nutrients may alter soil biochemistry processes such as microbial growth, activity, and thus soil multifunctionality (multiple soil functional enzymes) (Sun et al., 2022; Zhang et al., 2021). However, higher temperature may have also stimulated fungal growth which responsible for MPs degradation thus more additives released into the soil as discussed above. This would buffer against the positive effect of bio-degradable MPs on soil microbes, and therefore decreased the EMF under warming than ambient (Fig. 2d).

4.5. Factors mediating plant health under MPs pollution and warming

The random forest analysis confirmed that TDN, N_{min}, and AP are the main drivers of plant health (Fig. 5b). That is, soil with higher C and N availability generally allows for a diverse microbial community, ultimately accelerating biogeochemical cycles and improving nutrient cycling, which is beneficial for plant growth (Chen et al., 2020). A microenvironment with higher soil quality may provide suitable niches for microbial taxa (Jia et al., 2022), i.e., activate microbes and increase their richness and diversity, which in turn form microbial hotspots. These microbial hotspots are often highly related to SOM decomposition and nutrient recycling, further benefiting soil EMF. Thus, increases in soil quality can not only activate critical functions (e.g., SOM degradation, soil aggregate stability, nutrients cycling), but also advance the process of multiple ecosystems functions (Jia et al., 2022; Yan et al., 2021, 2022). However, this may exacerbate nutrient competition among microbial taxa and plants due to higher microbial growth rates, thus negatively affecting plant health.

As microplastics differ in shape, polymer type, and concentration, their effects on plant and soil may differ as a function of these properties (Lozano et al., 2020; Zhou et al., 2021a; Zhou et al., 2021b). More research should be conducted to evaluate the effect of shapes and concentrations on plant health and soil ecosystem functions, especially under various soils. In addition, the polymer chemistry, degradation rate, as well as its nutrient release should be taken into account when evaluating the effect of MPs on plant-soil-microbe interactions in the future.

5. Conclusions

Here, we found that conventional MPs (PE and PVC) had limited effects on soil biochemical properties and soil quality under ambient conditions, which may be due to the chemically stable polymer backbone structure of these plastics. By contrast, PHA provided an abundant source of C to support microbial growth, which contributed to nutrient cycling (C, N, P), and enhanced soil quality and ecosystem multifunctionality. However, the increased soluble C also induced microbial N immobilization and thus resulted in higher plant–microbe competition for essential nutrients (N, P), and consequently adversely affected plant health. Interestingly, most impacts of MPs mentioned above only occurred under ambient temperature, and were not present under warming temperature conditions. This suggested that high temperatures had a greater effect on plant health and masked the negative effect of MPs. This was explained by the decrease in chlorophyll content and stunted plant growth as a coping mechanism to heat stress. Overall, this study identifies the possible outcomes of MPs interaction with warming, given that agricultural stressors affect agroecosystem functioning cumulatively rather than individually, which in turn might affect ecosystem services and thus impact various aspects of human well-being.

Author contributions

Conceptualization, H. Z and G. N; data curation, G. N, R. J, Y. L and J. Z; formal analysis, G. N and J. Z; visualization, G. N, R. J and J. Z; founding acquisition, H. Z; writing—original draft preparation, G. N and J. Z; writing—review and editing, R, J, Y. L, R. B, H. Z, D. J, Z. Z; All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2022.116083.

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