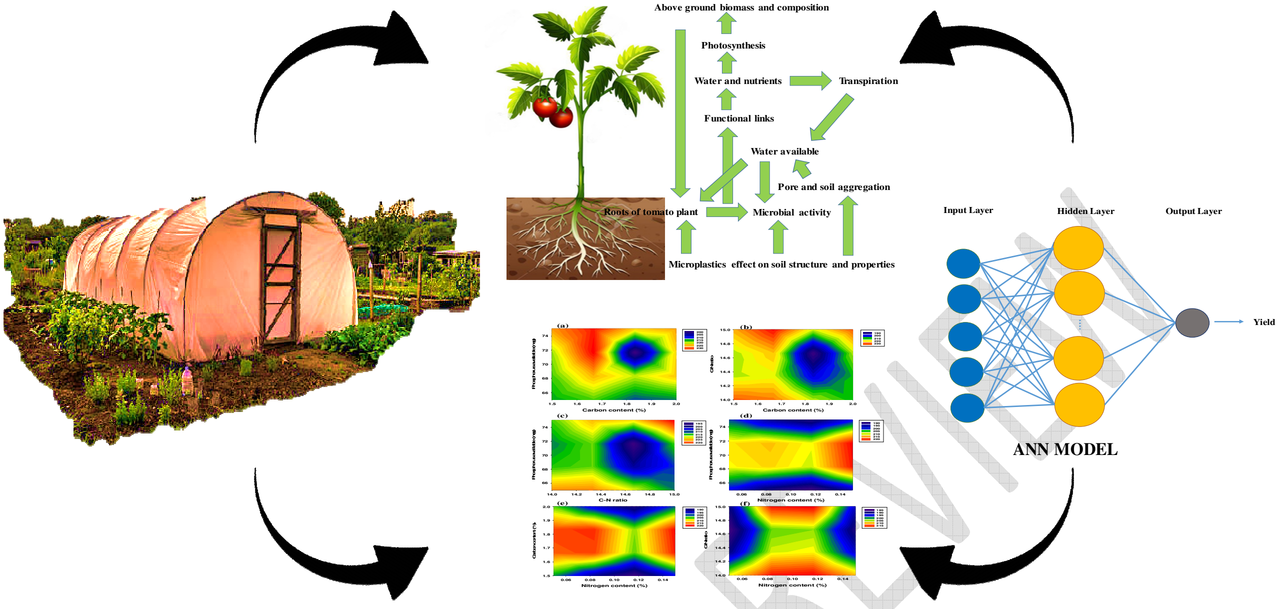
Effect of Polyethylene terephthalate microplastics on tomato plant: Experimental and AI modeling

Abstract:

Microplastics impacts on terrestrial ecosystem have gained attention in recent times, after about a decade of research being limited to aquatic systems. Although the impacts on soil physical characteristics and soil organisms are beginning to manifest, there is also a noticeable negative influence on plant growth and vitality. The Plant height, branches per plant, days to first fruit set, fruits per plant, fruit diameter, fruit weight (gm), and overall yield (q/hec) overall performances were explored. The tomato (*Solanum lycopersicum Linn*.) plant variety used in our study is Pusa ruby and the MPs used was Polyethylene terephthalate (PET). Different experimental parameters were also varied like MPs weight percentage (2%-5%), Nitrogen content (0.05 % -0.15%), Carbon content (1.5 %-2%), C-N ratio (14-15) and Phosphorus content (65-75). Furthermore, numerical modeling using artificial neural network (ANN) for validating the experimental results demonstrated an overall R^2 value > 0.99. Our results showed that overall yield of fruit was decreased and it has also effects on different plant morphological characteristics.



Graphical abstract:

1. Introduction

Plastics, which are synthetic organic polymers, have seen widespread use globally, with annual production reaching approximately 2.4 billion tons. These plastic materials can be easily transported over long distances through hydrodynamic processes and ocean currents. It has been reported that the ocean's surface contains an average of about 18,000 plastic items per square kilometer, with higher concentrations observed in regions such as the North Atlantic and Pacific Oceans. Over time, due to various factors like mechanical actions, biodegradation, and photo-oxidative degradation, plastic debris gradually breaks down into smaller particles, typically measuring less than 5mm, commonly referred to as MPs.

MPs have been shown to exhibit remarkable stability in water, potentially persisting for thousands of years due to their chemical durability. This newly recognized pollutant has been linked to substantial economic losses within marine ecosystems, estimated at around 13 billion dollars annually. As MPs increasingly infiltrate aquatic environments, they give rise to significant environmental and health concerns. Some of the key issues include MPs serving as efficient carriers of toxic organic chemicals due to their large surface area and hydrophobic nature. Persistent organic pollutants have been detected on the surfaces of MPs in coastal zones worldwide, including countries like the USA, the UK, South Africa, Brazil, and Hong Kong, with concentrations of substances like polycyclic aromatic hydrocarbons reaching as high as 24 mg/g. Additionally, MPs can adsorb various heavy metals, such as Zn, Cu, Pb, and Ag, and even nano-scale adsorbents like TiO_2 have been found on their surfaces. These pollutants can hinder the growth of aquatic species by impeding light penetration to some extent. Study on MPs contamination has primarily centered on oceans and aquatic ecosystems for the past decade, and the idea that terrestrial ecosystems might also be affected has emerged more recently (Rillig, 2012). Furthermore, MPs, due to their minuscule size, can be easily mistaken for food by marine organisms, leading to mechanical harm and decreased feeding efficiency. What's more concerning is that MPs carrying toxic substances play a significant role in the toxic effects on marine organisms. Studies have indicated that a mixture of MPs and pyrene can lead to a decrease in the activity of enzymes like acetylcholinesterase and isocitrate dehydrogenase. Additionally, the gene expression of fish can be altered after ingesting MPs loaded with organic pollutants. As a result, this presents a significant risk to human health when consuming contaminated seafood. MPs in aquatic environments are readily apparent and visible, in contrast to those in soil. Analytical techniques for assessing soil contamination by MPs have been established relatively recently (He et al.,2018). Unlike aquatic systems, the ongoing development of methods for analyzing MPs in soil remains a challenging and evolving process. Moreover, considering that the impacts observed in aquatic systems were largely associated with the presence of extra particles (potentially mistaken for food) and surfaces (capable of adsorbing and concentrating pollutants), this aspect appeared to be of lesser significance in the context of soil. Soil, being a particle-rich environment with an already extensive internal surface area, differs in this regard (Machado et al., 2018a; Jia et.al,2023). MPs have now been found in soils of several terrestrial ecosystems (Zhang & Liu, 2018). In these terrestrial environments, wildlife can ingest MPs as they forage for food, with birds mistakenly consuming them during soil insect hunting and larger animals ingesting them while grazing on contaminated vegetation. This ingestion poses risks to both individual organisms and the broader ecosystem. Once MPs find their way into the soil, they tend to remain, accumulate over time, and ultimately disrupt the normal functioning and biodiversity of soil ecosystems (Guo et al., 2019; Kumar et al., 2020). MPs also interact with the soil environment and alter the soil bulk density by affecting the stability of soil aggregates, which are building blocks of the soil structure (Rillig et al., 2021). The changes in soil fertility caused by MPs have indirect adverse impacts on plant growth. MPs influence soil fertility and subsequently affect plant growth by modifying root development and nutrient absorption (Zhou et al., 2023). Furthermore, MPs can accumulate in soils over time, affecting soil structure, nutrient cycling, and microbial communities. There's also the interconnectedness between terrestrial and freshwater ecosystems, whereby MPs entering freshwater systems can be transported downstream and deposited in terrestrial areas nearby. After being introduced to the soil surface through various means (Bl€asing and Amelung, 2018), multiple routes, such as biological processes, play a role in integrating MPs particles into the soil. (Rillig et al., 2017a, b; Huerta-Lwanga et al., 2017). The rate at which MPs break down in soil remains unknown, and the prevailing assumption is that these particles are durable and, consequently, will accumulate over time. (Rillig, 2012). Pusa Ruby is a determinate tomato variety developed by the Indian Agricultural Research Institute. It's known for its small to medium-sized, sweet, and tangy red fruits. This variety is adaptable to the Indian climate, resistant to some common tomato diseases, and has a good yield potential. It's typically grown in the Indian subcontinent as a summer crop, suited for a tropical or subtropical climate. These tomatoes are versatile and can be used fresh or for processing into sauces and curries. Despite ongoing research, there remain uncertainties about the long-term ecological consequences of MPs presence in terrestrial ecosystems, including potential disruptions to food webs and nutrient cycles. To address this concern, reducing plastic pollution at its source and implementing effective waste management practices are critical steps to mitigate the entry of MPs into terrestrial ecosystems.

2. MPs effect on different terrestrial ecosystems

It's crucial to acknowledge that MPs pollution is a tremendously intricate problem, marked by a broad array of plastic materials possessing diverse chemical compositions, additives, levels of persistence, surface characteristics, dimensions, and forms. These diverse characteristics result in different behaviors and effects within terrestrial ecosystems. For instance, a study by Machado et al. 2018b demonstrated that microfibers, when introduced at concentrations ranging from 0.05% - 0.40%, seem to exert a more pronounced impact on soil physical properties compared to beads, which were introduced at concentrations between 0.25% and 2.00%. Initially, MPs research primarily focused on polyethylene beads, mainly because of the reason that they are readily available. Yet, certain MP types remain unexplored, such as foams and various composite materials. Similar hypotheses could potentially be formulated, with the availability of more data, for different chemical compositions and other facets of MPs, particularly their surface characteristics.

3. Materials and methods

The detailed physicochemical characteristics of this soil have been carried out at the experimental facilities of Regional Research Station, Agwanpur, Saharsa (25°55′32” N, 86°33′36” E; Saharsa, Bihar) on March 4, 2023, including information such as a nitrogen content of approximately 0.1%, carbon content of around 1.79%, a C-N ratio of about 14.87, pH level of approximately 7.4, and available phosphorus content of approximately 72 mg per kg. Subsequently, we exposed the soil under investigation to polyethylene terephthalate PET MPs for approximately two months. The tomato plants were allowed to grow for an additional approximately 1.5 months, during which we conducted a comprehensive analysis of various indicators related to the health of both the soil and the plants.

3.1. MPs used in the study

In this study, primary MPs made of PET were the main focus. The PET used in the study was obtained from Sigma Aldrich. To ensure purity, the PET underwent a rinsing process with 1 mol/L HCl to remove impurities and was kept away from light before experiments. These industrial pellets were then processed by grinding machine after being made brittle with liquid nitrogen. Subsequently, the ground materials were dried and sieved through a 1 mm sieve. Laser diffraction analysis revealed that the most prevalent size range for the PET particles was between 100 to 200 µm, with a median size of 154 µm.

3.2. MPs Addition to the Soil

To reduce the risk of microbial contamination, MPs underwent a microwave treatment lasting 5 minutes, it’s temperature did not reach the melting points during the process. An initial test confirmed that Petri dishes containing MP particles subjected to microwave treatment did not exhibit visible signs of microbial growth over approximately 2 months at around 20°C.

Subsequently, these MPs were promptly introduced into freshly collected soil. PET was added at a concentration of 2% relative to the fresh weight of the soil. The initial soil moisture content stood at approximately 12.6 ± 0.5%. These levels of MPs can be considered ecologically relevant for soils exposed to substantial human activity, as they were determined based on prior experiments, which had observed noticeable alterations in the soil's biophysical environment. The plastic and soil was mixed by stirring them together in a glass beaker using a metal spoon, involving approximately 500 grams of experimental soil and a duration of 20 minutes. As a control, a treatment was incorporated where no plastic was added but underwent equivalent stirring. It should be noted that quantifications of plastics were not conducted due to the absence of an established methodology for extracting and measuring MPs amount in soils with irregular compositions and shapes.

3.3. Soil and tomato plants exposure to MPs

The soil samples, each weighing 200 grams, were placed into 200 ml glass beakers that had been earlier sterilized using a microwave. To enhance the statistical accuracy and precision of our study, we increased the number of replicates in the control group, ensuring that all samples treated with MPs could be compared to controls. These prepared beakers, containing soil treated with MPs were covered with aluminum foil. Subsequently, they were placed in the greenhouse located at Bihar, where the temperature was maintained at approximately 22 ± 3 °C, for a duration of around 2 months. During this initial incubation period, the soil samples were kept in darkness, and their moisture levels were carefully monitored, with watering occurring approximately three times a week to maintain a high moisture level. Specifically, the soils were brought to 85% of their water-holding capacity each time the moisture level dropped to around 25%. Distilled water was gently sprayed onto the soil surface for watering. On July 29, 2023, signifying the end of this incubation duration, nine seedlings derived from tomato seeds that had been sterilized on the surface were added to half of the beakers. All beakers were then retained in the greenhouse for an extra period of roughly 1.5 months, until September 15, 2023. During this time, they were watered every two days to maintain moisture levels at 55% of the water-holding capacity. Consequently, 14 replicates were tested for soil with plants in the control group, 14 replicates for soil without plants in the control group. The overall mechanism that generally takes place is shown in Fig.1.

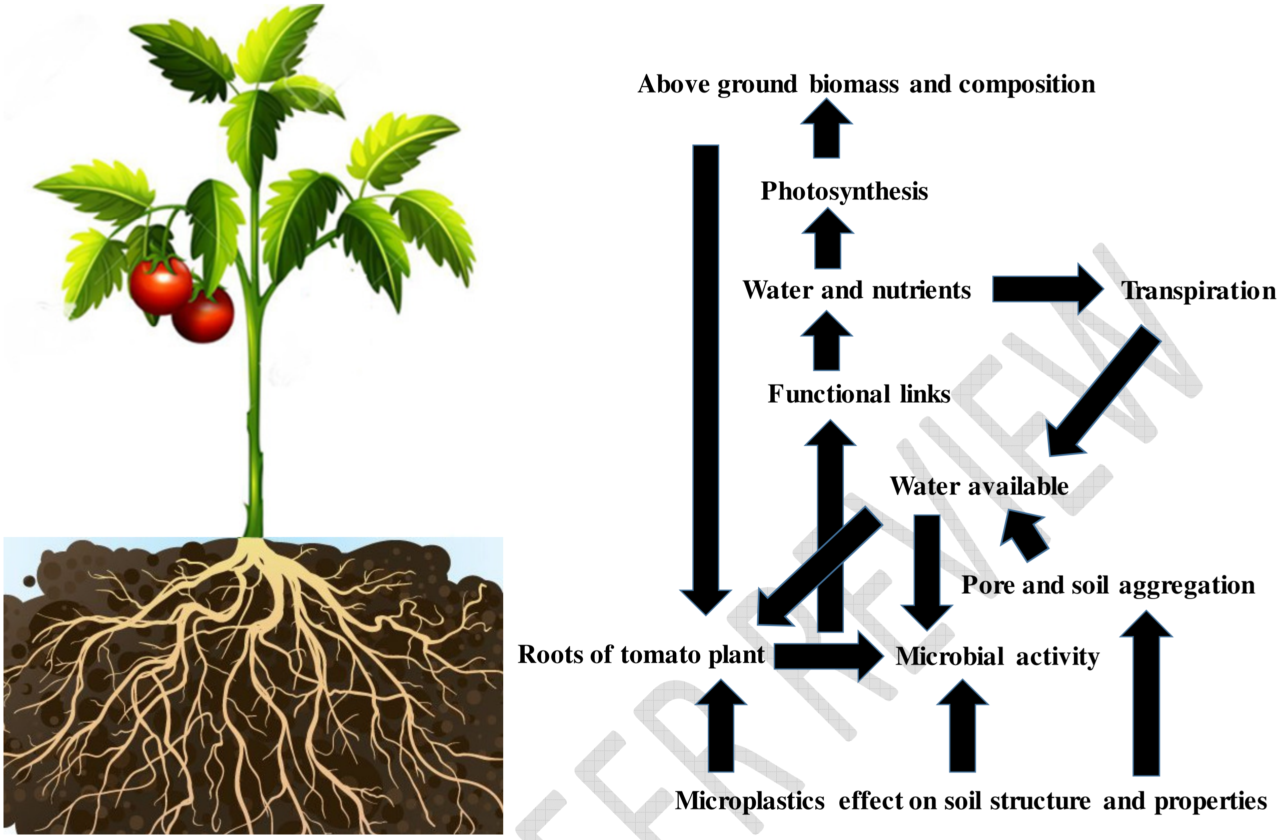


Fig.1. Diagram of the overall mechanism involved during process.

3.4. Proxies of Soil and Plant Health

Experiments were conducted to evaluate evapotranspiration on July 25, 2023 by fully saturating the soils with distilled water, reaching 100% of their water holding capacity, and monitoring weight changes over 72 hours. The reductions in weight were then converted into water loss, with a conversion rate of approximately 1 gram being equivalent to 1 milliliter of water loss. It is worth noting that, even though we consistently refer to this process as evapotranspiration throughout our manuscript, the majority of water loss observed was primarily due to evaporation.

At the time of harvest, we measured the soil volume to calculate bulk density. Additionally, we collected surface soil samples, each weighing approximately 0.5 grams and microbial activity was evaluated by measuring the hydrolysis of fluorescein diacetate (FDA) with three analytical replicates. Soil structure was evaluated following the methodology (Machado et al.,2018). Specifically, the entire soil sample underwent a careful process of filtration through a series of layered sieves, each with varying mesh sizes (4000, 2000, 1000, and 200 μm). Fresh roots were washed with distilled water and then subjected to scanning using a photo scanner. Various plant characteristics, including plant height, branches per plant, days to first fruit set, fruits per plant, fruit diameter, fruit weight, and yield, were also measured. Furthermore, the dry masses of both the plant's aboveground and belowground tissues were ascertained following a 48-hour drying period in an oven set at 60°C.

4. Results and discussions

4.1. Effects on plants

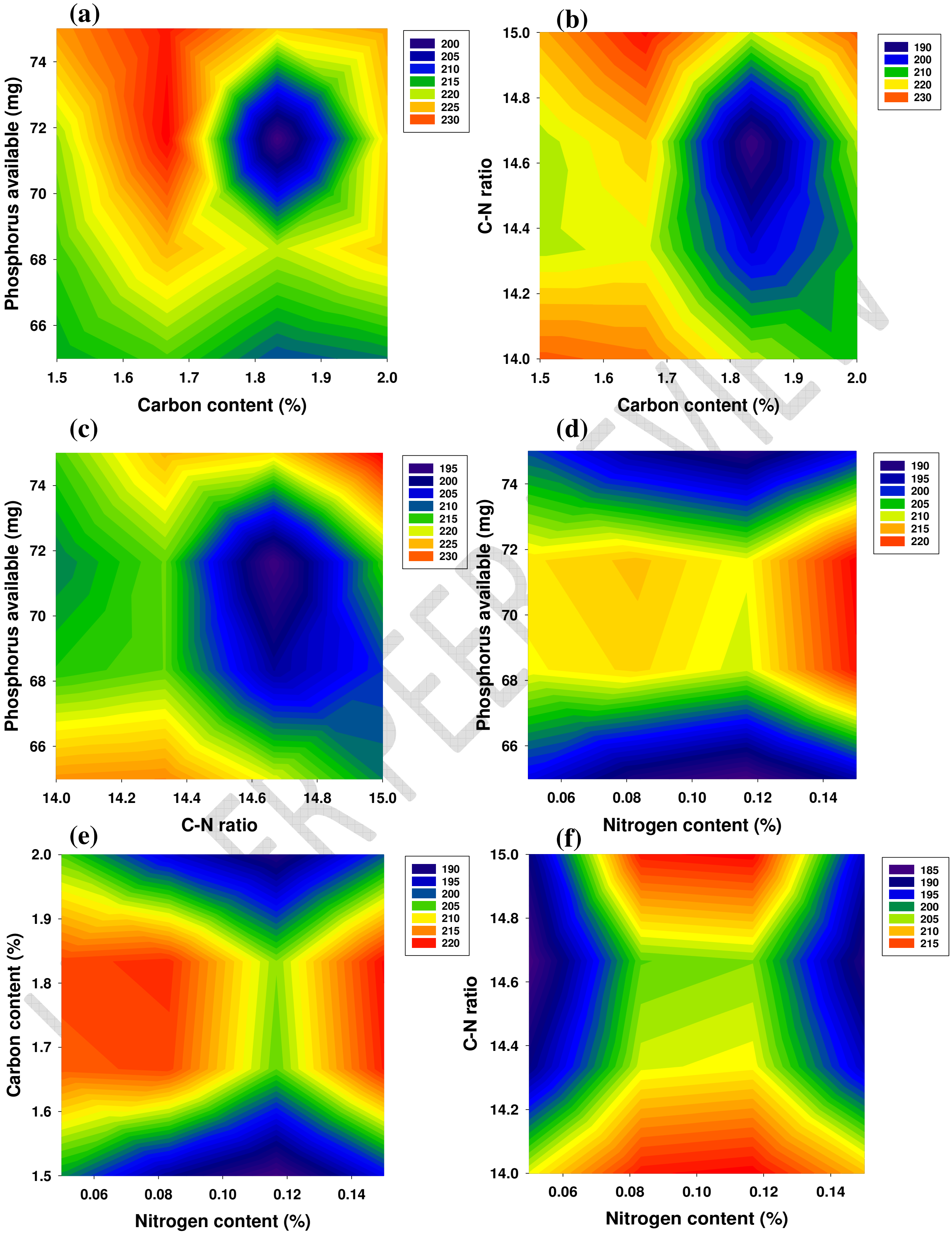
To comprehensively grasp the impact of MPs on plant performance, it's crucial to consider various potential mechanisms that may come into play (as depicted in Fig. 1). We believe that multiple mechanisms are likely at work, and their significance may vary depending on the type of soil properties involved.

4.2. Altered soil structure

MPs can be conceptually regarded as contaminants that impact the physical properties of soil, as suggested by Machado et al.,2018a. Primary data indicates that microfibers, as highlighted in Machado et al. (2018b), have indeed been associated with a reduction in soil bulk density. This reduction could directly result in lower penetration resistance for plant roots, improved soil aeration, and consequently, enhanced root growth (Zimmermann and Kardos, 1961). However, there are other potential effects to consider. For instance, research has demonstrated that plastic films, when present in the form of 2, 5, and 10 mm size fragments and added at concentrations ranging from 2% to 5%, can create channels that facilitate water movement, ultimately leading to increased water evaporation (Wan et al., 2019). This may potentially lead to soil desiccation, posing potential adverse outcomes for plant performance. Changes in soil structure can trigger various secondary effects. It's inevitable that a significant alteration in a fundamental soil parameter like soil structure will induce shifts in the composition of soil microbial communities, even though predicting the precise nature of these shifts and their functional implications can be challenging. If such alterations impact root symbionts, including mycorrhiza and nitrogen-fixing organisms, it could subsequently influence plant growth. Additionally, modifications in overall soil structure can affect the process of soil aggregation, as observed in Machado et al. (2018b), In a particular field study, it was observed that microfibers had an adverse effect on soil aggregation, potentially disrupting it. Conversely, there is the potential for beneficial effects on soil aggregation if microfibers act to entwine soil particles, assisting in the formation of soil aggregates. Since soil aggregation plays a role in determining soil structure, these alterations can impact soil aeration and the growth of plant roots, as previously mentioned.

4.3. Nutrient immobilization

Plastic particles contain a substantial amount of carbon, as highlighted in study of Rillig's, 2018. A significant portion of this carbon is relatively inert due to the slow decomposition of plastic materials. Over time, this inert carbon will gradually break down, resulting in microbial immobilization, primarily due to the exceptionally high carbon-to-nitrogen (C-N) ratio. It's important to note that this process occurs at a pace that is likely inconsequential in terms of biological effects. However, it's anticipated that such effects would be more significant when dealing with MPs materials characterized by lower persistence, such as biodegradable plastics. In the study, variations in MPs weight percentages were explored, and it was observed that changes in the C-N ratio had negligible effects on fruit yield. The contour plot for correlative effects between various factors affecting yield is shown in Fig.2.


(a)
Phosphorus available (mg)
74
72
70
68
66
1.5
1.6
1.7
1.8
1.9
2.0
Carbon content (%)
200
205
210
215
220
225
230
(b)
C-N ratio
15.0
14.8
14.6
14.4
14.2
14.0
1.5
1.6
1.7
1.8
1.9
2.0
Carbon content (%)
190
200
210
220
230
(c)
Phosphorus available (mg)
74
72
70
68
66
14.0
14.2
14.4
14.6
14.8
15.0
C-N ratio
195
200
205
210
215
220
225
230
(d)
Phosphorus available (mg)
74
72
70
68
66
0.06
0.08
0.10
0.12
0.14
Nitrogen content (%)
190
195
200
205
210
215
220
(e)
Carbon content (%)
2.0
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1.7
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0.08
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Nitrogen content (%)
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C-N ratio
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Nitrogen content (%)
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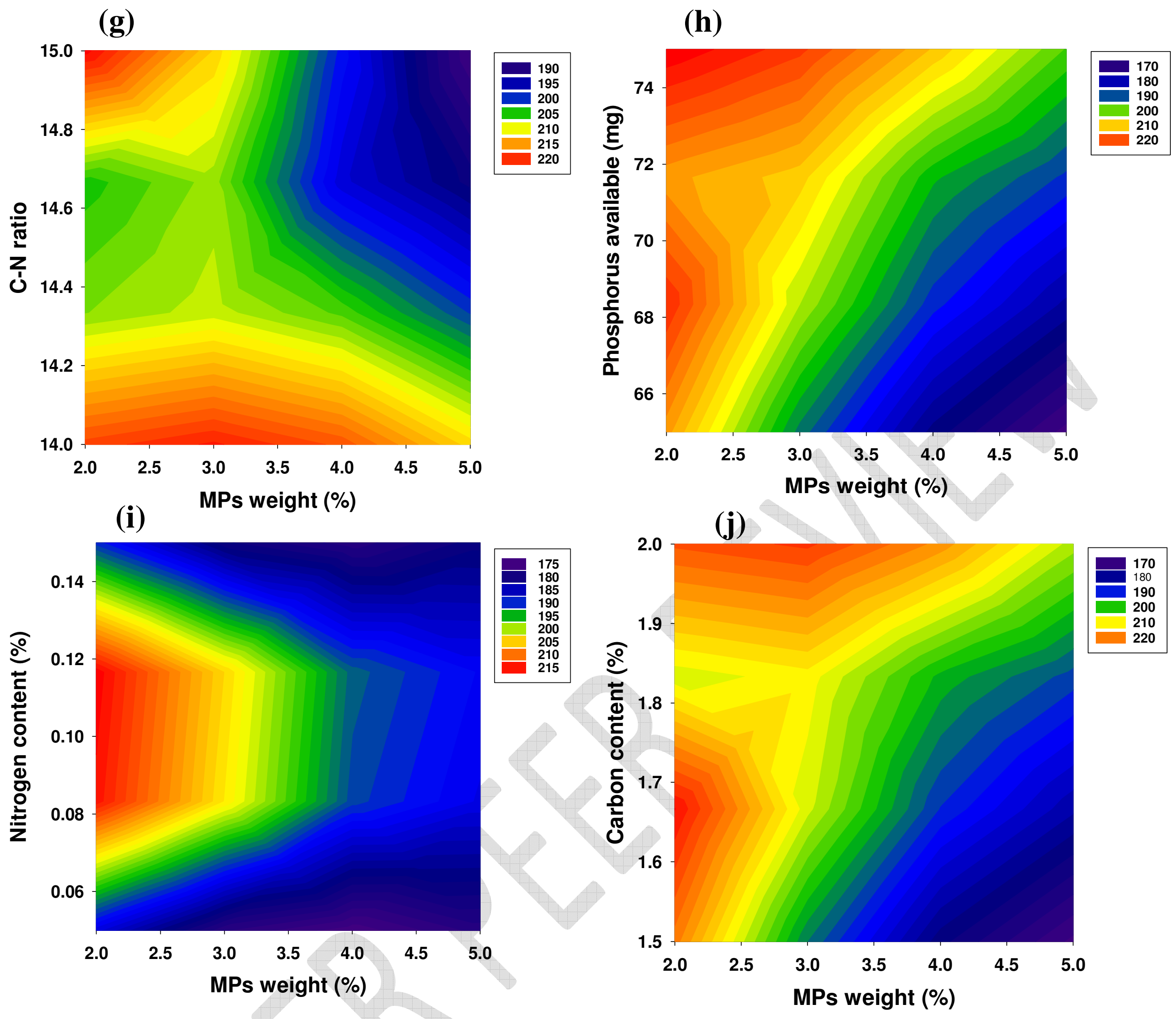

Figure 2. Contour plot for correlative effects of (a) Carbon content and phosphorus available; (b) Carbon content and C-N ratio (c) C-N ratio and phosphorus available (d) Nitrogen content and phosphorus available (e) Nitrogen content and carbon content (f) Nitrogen content and C-N ratio (g) MP weight and C-N ratio (h) MP weight and phosphorus available (i) MP weight and Nitrogen content (j) MP weight and Carbon content

4.4. Contaminant adsorption and transportation

MPs can introduce unique surface properties to soil, such as hydrophobic characteristics. These properties could lead to the enrichment and potential long-term stability of specific contaminants, especially those with traits like hydrophobicity. Substances that might already be present in MPs before they enter the soil, possibly introduced during their manufacture, have the potential to be carried into the soil along with these MPs particles. These toxic substances, whether adsorbed onto the surface or contained within the MPs particles in the soil (Galloway et al., 2017), can have adverse effects on plant roots or their symbiotic relationships, potentially resulting in negative impacts on plant growth parameters like fruit diameter, yield, and plant height. On the other hand, the attachment of contaminants to the surfaces of MPs may make other pollutants less available to soil organisms and plants, potentially providing a protective effect. This phenomenon has been observed in aquatic environments (Kleinteich et al., 2018; Rehse et al., 2018), and similar effects may also be relevant in terrestrial soils. Consequently, there is presently significant uncertainty regarding whether the presence of MPs will enhance or diminish the effects of pollutants in the environment

4.5. Toxicity due to MPs

As MPs size decreases, it is theorized that the effects on organisms become more chemical and potentially toxic in nature, rather than purely physical (Yang et al.,2017). While it is not anticipated that microsized particles will be taken up by plant roots, the situation differs for nanoplastic particles. Detecting nanoplastic particles in soil has posed difficulties because existing extraction and measurement techniques often fail to identify them or lack the capability to offer size-specific data. The primary site of contact and a potential barrier for nanoplastic uptake would likely be the root's rhizodermis. Although the mechanisms that control the uptake of nanoparticles by plants are not well comprehended, it is recognized that particles at the nano-scale have the potential to enter plant roots and result in adverse consequences, including changes to cell membranes, intracellular substances, and the induction of oxidative stress, as suggested by Navarro et al. (2008). In the case of a crop plant, this scenario could suggest that plastics might find their way into the plant's edible parts, which are intended for consumption by humans or livestock, ultimately entering the food chain., as discussed in Bouwmeester et al. (2015). Nonetheless, Awet et al. (2018) observed negative short-term impacts of polystyrene Nano plastics on enzymatic processes and soil microbial activity, even when plants were not present.

4.6. Soil microbial community and root symbionts

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4.7. Plant community-level effects

It was quite evident that different plant species within a community could experience varying degrees of impact when exposed to MPs, either in mixed forms or as individual types. This suggests that MPs have the capacity to impact both the diversity of plants and the makeup of plant communities through various underlying mechanisms. For instance, the properties of a plant community are intimately linked to soil structure, including factors such as soil aggregation, (Pohl et al., 2012; Peres et al.,2013). Hence, the notable changes in soil structure caused by different types of MPs have the capacity to bring about changes in the composition of plant communities. For example, plastic films may lead to increased evaporation of soil water (Wan et al., 2019), This could result in more severe drought conditions, ultimately promoting the proliferation of plant species that are adapted to withstand drought within a community.

Moreover, the soil microbial community has a significant role in influencing the composition, productivity, and diversity of plant communities (Wagg et al.,2014; Powell & Rillig , 2018). Changes in the makeup of soil microorganisms or symbiotic organisms that colonize plant roots after the introduction of microplastics can additionally impact the composition of the plant community. This is because soil microbial diversity and root-colonizing symbionts often have positive effects on plant diversity, as highlighted in previous research The impacts on plant communities are more likely to become evident in regions experiencing greater levels of MPs pollution. making them a potential concern, particularly in proximity to agricultural fields or urban areas.

4.8. Effect on plant morphological characteristics

In our research, we employed the Pusa Ruby variety of tomato plants, and upon their interaction with soil containing MPs particles, various alterations were observed in the morphological characteristics of the plants as shown in Table 1. As we varied the percentage of MPs weight up to 5%, we noted significant changes in plant performance. The yield (q/hec) declined from 300 to 188, while plant height decreased from 106.9 cm to 67.37 cm. Additionally, the number of branches per plant decreased from 14.8 to 6.2, the time required for fruit set increased from 31.3 to 59.3 days, and the number of fruits per plant decreased from 17.8 to 7.4. Furthermore, the diameter of the fruits reduced from 4.8 cm to 2.3 cm, and the fruit weight declined from 77.6 grams to 39.3 grams. Consequently, it is evident that PET MPs have a significantly detrimental impact on tomato plants.

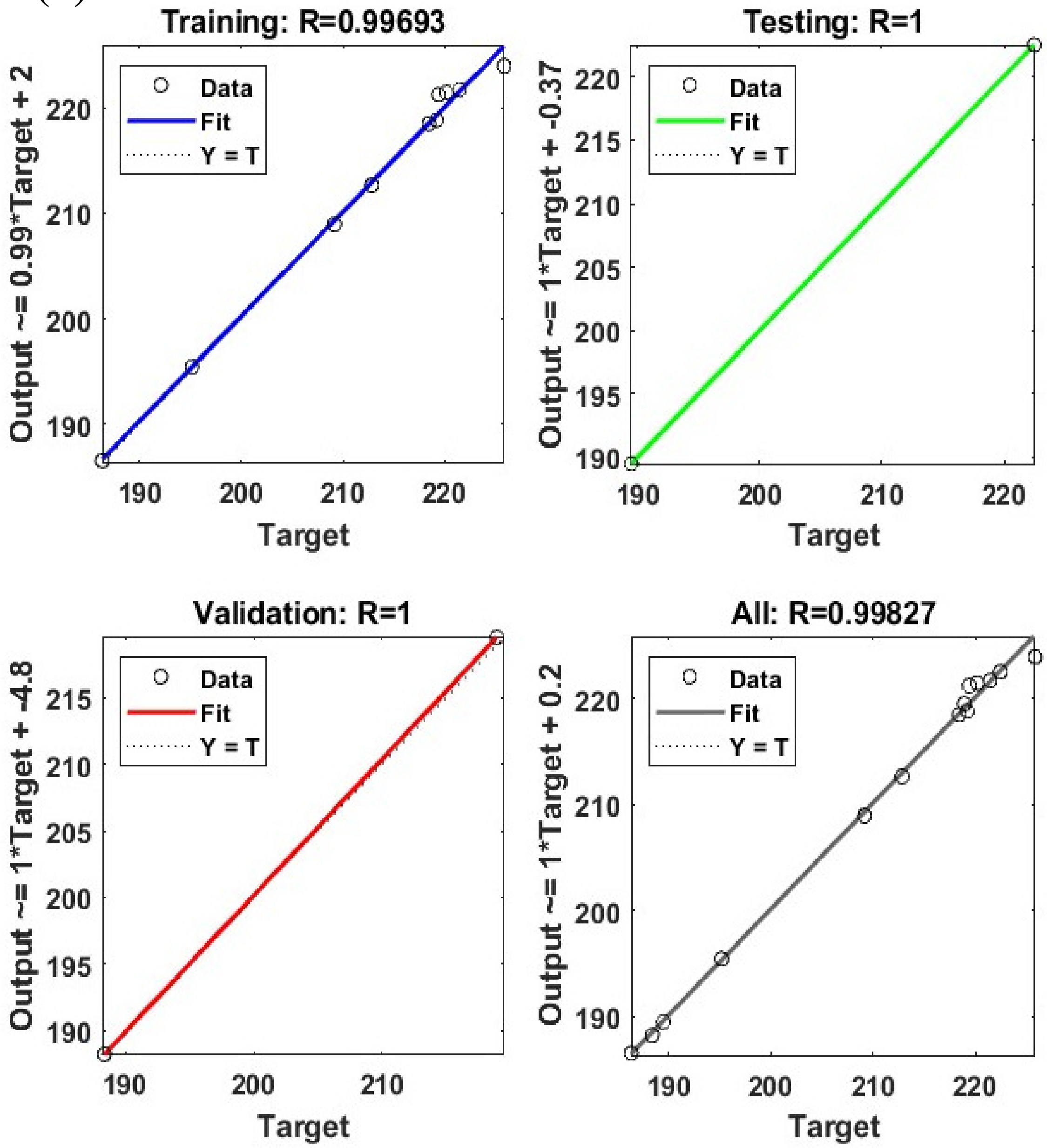
MPs weight percentage (%)	Variety	Plant height (cm)	Branches/plant	Days to first fruit set	Fruits/plant	Fruit diameter	Fruit wt (gm)	Yield (q/hec)
2	**Pusa Ruby**	**96.37**	**9.45**	**45.31**	**11.38**	**3.37**	**57.36**	**220.16**
3		**86.19**	**8.28**	**50.31**	**9.38**	**3.12**	**51.36**	**209.16**
4		**79.37**	**7.28**	**55.31**	**8.38**	**2.67**	**47.36**	**195.16**
5		**67.37**	**6.28**	**59.34**	**7.48**	**2.37**	**39.36**	**188.38**

Table.1 Effect on different morphological characteristics of the plants

5. ANN modeling

Typically, an ANN is composed of three layers: input, hidden, and output layer. This study investigated the impacts of five independent variables on final yield, the neural network’s input layer was designed with five neurons. As the objective of the neural network was to perform a regression task, the output layer was composed of only one neuron. Furthermore, hidden layer neurons were altered from 1 to 20 to optimize the layer configuration, and the resulting RMSE values were recorded (Fig. 3b). The model was created based on the experimental dataset and was trained using 70% of the data, while 15% was reserved for testing and another 15% for validation. Throughout the training process, the model with a high R^2 and low RMSE value was used as the primary criterion to decide when to stop the training. The optimum model with the least RMSE value was obtained using 4 neurons in the ANN model. For final yield, the ANN model exhibited an R^2 value greater than 0.99 and the tansig transfer gave best result as shown in Fig.3 a,b.

(a)



Training: R=0.99693
Output ~= 0.99*Target + 2
Target
190
200
210
220
Data
Fit
Y = T
Testing: R=1
Output ~= 1*Target + -0.37
190
195
200
205
210
215
220
Target
Data
Fit
Y = T
Validation: R=1
Output ~= 1*Target + -4.8
190
195
200
205
210
215
Target
Data
Fit
Y = T
All: R=0.99827
Output ~= 1*Target + 0.2
190
200
210
220
Target
Data
Fit
Y = T

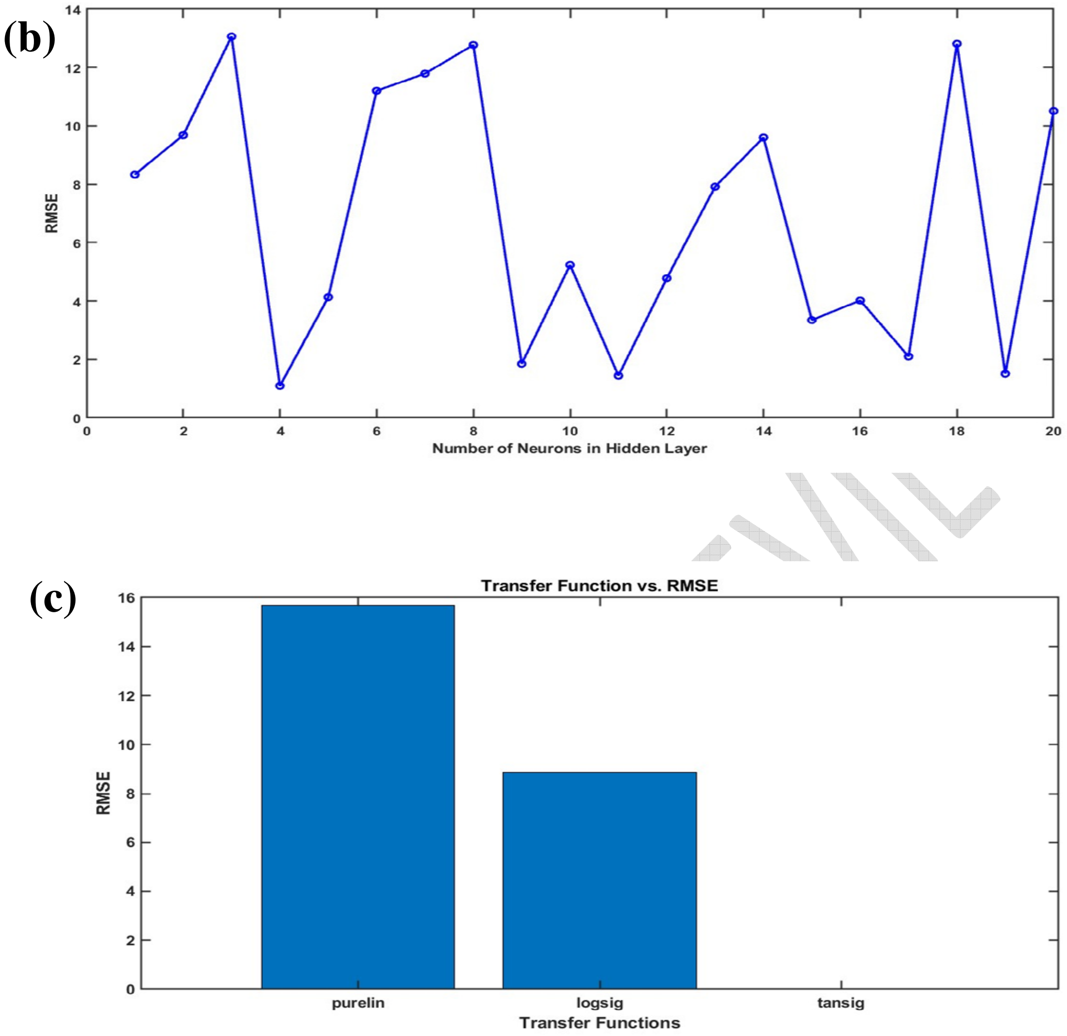


Fig.3. (a) ANN regression curve (b) Number of neurons in hidden layer vs RMSE (c) Transfer function vs RMSE

6. Conclusions

MPs represent a concerning class of contaminants and, given their widespread presence, should be considered a contributing factor to global environmental change. Currently, our understanding of how this global change factor affects plants is extremely limited. In this study, several potential mechanisms were outlined through which these materials could influence plant performance. It's important to note that some of these mechanisms may lead to positive effects on root development and plant growth, while others could have detrimental consequences.

The experiments were conducted where various parameters, including the percentage of MPs weight (2% - 5%), nitrogen content (0.05% - 0.15%), carbon content (1.5% - 2%), C-N ratio (14 - 15), and phosphorus content (65 - 75) were investigated. Our findings revealed an overall decrease in fruit yield, along with notable effects on various morphological characteristics of the plants. It's important to acknowledge that the magnitude of these effects is likely to vary depending on the plant species under consideration, potentially leading to shifts in plant community composition and, perhaps, alterations in primary production. In summary, our study demonstrated a reduction in the overall productivity of tomato plants, resulting in a significantly lower final yield compared to the initial yield, primarily due to the influence of MPs.

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