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Microplastics detection in agricultural soil combining 3D Laser Scanning Confocal Microscopy with machine learning

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Microplastic (MP) contamination in agricultural soils is increasingly linked to low-density plastics originating from plasticulture (e.g., mulching) and from MP-contaminated organic fertilisers such as compost and sewage sludge. Although these entry pathways are well documented, robust quantification of MP in soil remains challenging and time-consuming. Established microscopic-spectroscopic approaches (μ -Raman, μ -FTIR) are highly effective in aquatic matrices but require intensive soil sample preparation because soil organic matter (SOM) interferes with polymer identification. Many soil protocols rely on density separation with high-density salt solutions (e.g., ZnCl_2) and chemical oxidation to remove SOM with hazardous and corrosive reagents that can modify MP in relevant ecotoxicological parameters like size, shape, and surface properties. Additionally, MP surface quantification is still rarely integrated into routine analysis, despite its relevance to toxicity. To address these limitations, this study developed and rigorously evaluated a hazard-free workflow for MP detection and surface property quantification in contaminated agricultural soils without the need for SOM removal. The key advance is automated MP detection in the presence of SOM, while enabling 3D surface property quantification by surface roughness-related descriptors. The workflow combines (i) sample preparation for 25 g soil (<2 mm fraction) using physical dispersion, density separation with ultrapure water, and freezing-based extraction technique, with (ii) high-resolution 3D Laser Scanning Confocal Microscopy (3D LSM; Keyence VK-X1000, Japan) and (iii) machine-learning-based data analysis using a supervised Random Forest classifier with respecting optical, laser and height values. The 3D LSM scans a 25 mm diameter filter with a minimum pixel size of 2.7 μm and a height resolution of 4 μm . The Random Forest classifiers enable reliable identification even when soil contact modifies the apparent colour of MP particles and avoid watershed segmentation in the postprocessing. The workflow outputs MP particle counts alongside detailed size distributions, 3D shape, and quantification of surface properties. Performance was evaluated using three agricultural topsoils spanning contrasting textures (sand, loam, silt) spiked with representative polymers: transparent polypropylene, transparent low-density polyethylene (LDPE), and black LDPE particles across three size fractions <53 μm , 53-100 μm , and 100-250 μm , plus fibres (1000 μm length). The method reliably detected both transparent and black MP $\geq 53 \mu\text{m}$ in soils with low particulate organic matter content, achieving a mean recovery rate of $80\% \pm 28\%$. Transparent MPs were robust

against low particulate organic matter, whereas black MPs and fibres were more sensitive. MPs <53 µm were consistently underestimated, regardless of SOM presence or soil texture, indicating current limitations driven by physical dispersion during sample preparation and size-dependent background correction. Four parallel samples (4 x 25 g; 100 g total soil) can be processed within three days, from preparation to analysis, enabling rapid throughput without the use of hazardous substances. As density separation is performed with ultrapure water, the workflow is currently most suitable for low-density polymers. Overall, this hazard-free 3D LSM–Random Forest workflow provides a scalable and automated tool for screening and characterising low-density MPs in agricultural soils, generating quantitative datasets that support ecotoxicology-relevant assessments and complement existing laboratory approaches.