Journal of Environmental Quality

TECHNICAL REPORTS

Emerging Contaminants

Sodium hypochlorite as an oxidizing agent for removal of soil organic matter before microplastics analyses

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Assigned to Associate Editor Jun-Jian Wang.

Abstract

The omnipresence of microplastics (MPs) across Earth's surface has raised concerns about their environmental impact and created an urgent need for methods to identify them in complex soil and sedimentary matrices. However, detecting MPs in the O horizons of soils is difficult because plastic polymers share many physical and chemical properties with natural soil organic matter (SOM). In this study, we assessed whether sodium hypochlorite (NaOCl), a reagent that can oxidize SOM and simultaneously preserve mineral constituents, can be used for MP analysis and characterization in soil environments. In addition, we scrutinized how factors such as MP size, polymer type, extraction methods, and soil matrix affect the recovery of microplastic particles. We used both hydrophobic and density-dependent separation methods to assess the effects of our oxidation treatment on the recovery of MP. We observed that NaOCl effectively removed SOM without greatly altering the surface properties of resistant MP polymers (polypropylene, polylactic acid, low-density polyethylene, and polyethylene terephthalate), which were characterized using scanning electron microscopy and Fourier-transform infrared spectroscopy after SOM removal. The NaOCl treatment caused some chlorination and formation of additional C–OH bonds on polymer surfaces, which likely contributed to the reduced efficiency of the hydrophobic-based (oil) extraction. We conclude that NaOCl treatment can improve detection of MPs in SOM-rich soil and that recovery of MPs from soils is influenced by MP size, polymer type, extraction method, and soil type, which makes it challenging to develop a universal analytical method.

1 | INTRODUCTION

Microplastics (MPs; i.e., synthetic polymers <5 mm in diameter) are pervasive in modern ecosystems (Arthur et al., 2009). Littering, sewage sludge addition, dust deposition, and direct release of plastic waste into terrestrial ecosystems are potential vectors of MPs to soils (Horton et al., 2017; Tudor et al., 2019). Long-range atmospheric transport and ocean currents can also deliver MPs to remote terrestrial (Allen et al., 2019) and ocean ecosystems, including the polar regions and ocean basins (Barnes et al., 2009; Oigman-Pszczol & Creed, 2007; Tekman et al., 2020; Van Cauwenberghe et al., 2013). The imminent dispersal of microplastics across Earth's surface highlights the importance of studying the fate of these particles but doing so requires the development of time- and cost-effective methods to detect synthetic polymers in aquatic and terrestrial ecosystems (He et al., 2018). To date, research on the fate of artificial polymers in terrestrial systems has focused mainly on agricultural soils with relatively low soil organic matter (SOM), and these soils are the most common recipients of MP-bearing sludge or wastewater (Liu et al., 2018; Zhang et al., 2020).

The O horizons of pristine boreal forest soils are heavily enriched with SOM, ranging from ~40 to nearly 100% (Feng et al., 2016). This natural organic matter is a major obstacle when analyzing MPs in environmental matrices (Lu et al., 2021). The most common methods for isolating MPs from soils and sediments depend on a pre-analysis separation step where the polymer density (Coppock et al., 2017; Imhof et al., 2012), hydrophobicity (Crichton et al., 2017), or electrostatic properties (Felsing et al., 2018) are used to extract these MP particles from the soil or sedimentary matrix. All three separation methods have proved functional when applied to samples dominated by minerals that provide a matrix with vastly different properties than the polymers of interest. However, degraded plant materials (i.e., humus) and roots have densities and electrostatic properties comparable to most artificial polymers, and separating MPs from organic matter is difficult (Blasing & Amelung, 2018). As SOM mixes with polymers during the separation step, it makes the MPs more difficult to detect visually (Radford et al., 2021), and organic coatings on MPs can mislead microscopic (Hidalgo-Ruz et al., 2012) and spectroscopic (Mai et al., 2018) analyses. These observations collectively call into question our ability to accurately quantify MPs in soil and sedimentary archives enriched in natural organic matter.

Common methods to remove SOM rely on combustion at temperatures approaching ~550 °C (Kettler et al., 2001). However, these methods are rendered useless in the context of MPs because plastics melt at ~100–300 °C. Similarly, wet chemical treatments (i.e., acid, alkaline, or oxidizing agents) that are also commonly used to remove organic matter from

Core Ideas

- Sodium hypochlorite (NaOCl) induces a chlorination of the polymer surface.
- NaOCl at room temperature reduces interference from humus during microplastic separation.
- NaOCl treatment can remove organics but preserve polymers and soil minerals.
- Recovery of microplastics depends on polymer type, size, extraction method, and soil type.

soils risk degrading the polymer(s) of interest and/or causing color fading that may hamper MP identification by morphology (Blasing & Amelung, 2018). For example, polyethylene terephthalate (PET), polycarbonates, and cellulose acetate (CA) are severely degraded by sodium and potassium hydroxide (Dehaut et al., 2016; Hurley et al., 2018). Similarly, polyamide (PA), polypropylene (PP), and polystyrene (PS) undergo substantial degradation and discoloration after treatment with hydrochloric acid, nitric acid, or hydrogen peroxide (Cole et al., 2014; Dehaut et al., 2016; Nuelle et al., 2014; Wang et al., 2018). In other words, chemically oxidizing SOM without compromising the integrity of MPs in soils and sediments remains a challenge. Enzymatic digestion offers one alternative that has been successfully applied (Cole et al., 2015; Löder et al., 2017; von Friesen et al., 2019), but their high costs make them unfavorable for scaling up replicate numbers or sample quantities. Moreover, although digestion methods (e.g., a pretreatment step for MP analysis) have been developed for various environmental matrices, such as mangrove sediments (Duan et al., 2020), biota (Enders et al., 2016), and sludge from sewage treatment plants (Hurley et al., 2018), we still lack similar studies based on SOM-rich soils.

When searching for MPs in SOM-rich matrices, hydrogen peroxide (H_2O_2) or Fenton's reagent are the preferred oxidizing agents (Hurley et al., 2018; Radford et al., 2021). However, because H_2O_2 and Fenton's reagent are strong modifiers of inorganic soil phases (Mikutta et al., 2005), they are unsuitable for assessing mineralogical composition of soil (e.g., mineralogy, cation exchange capacity, and mineral specific surface area). Highly reactive secondary minerals that are rapidly consumed by H_2O_2, such as iron oxides, contribute to the persistence and preservation of organic matter (Feng et al., 2015; Lalonde et al., 2012; Schmidt et al., 2011; Torn et al., 1997), making it plausible that they also affect the breakdown of artificial polymers. In other words, there is an urgent need to find a chemical agent that can remove SOM without modifying inorganic and MP properties and therefore allows

study of polymer–mineral surface interactions. With the negative effects on inorganic phase integrity caused by H_2O_2 and Fenton's reagent in mind, sodium hypochlorite (NaOCl) has been put forward as an alternative oxidant suitable for SOM removal because it preserves poorly crystalline minerals when applied at pH <9.5 (Lavkulich & Wiens, 1970; Mikutta et al., 2005). In addition to preserving the integrity of soil minerals, NaOCl oxidations do not require additional heating. Despite these potential benefits, NaOCl has never been evaluated for its SOM removal potential and MP preservation efficiency in soils with high SOM content. However, it is unknown to what extent oxidation and chlorination induced by NaOCl affects polymer surfaces and hampers methods used to detect MPs in soils (i.e., the ability to separate and identify MPs). Indeed, if alterations of physical and chemical characteristics of plastic remain negligible, NaOCl could be used as a reagent and ultimately improve our ability to assess the interaction between MPs and mineral constituents in soils.

In this study, we hypothesize that NaOCl can improve recovery of MPs from SOM-rich matrices without jeopardizing the chemical or morphological signature of the polymer of interest. To test this hypothesis, we added a NaOCl oxidation step to protocols previously developed for MP extraction from sedimentary matrices (Coppock et al., 2017; Crichton et al., 2017) and assessed the effects of the oxidation process on MP recovery rates and polymer surface properties using XPS and FTIR.

2 | MATERIALS AND METHODS

2.1 | MPs preparation of soil samples

To evaluate the ability of two common methods to recover MPs from different types of matrices, we used soil derived from a podzol (B horizon) and from an organic-rich histosol. The two soil matrices differed greatly in SOM content (i.e., 42% for the histosol and 1% for the podzol). The sampled soils were judged free from inputs of artificial polymers because they were sampled from deep soil layers and therefore had no direct contact with atmospheric inputs. A detailed description of the sites is provided in the Supplemental Information.

To allow assessment of eventual size-dependent method biases, we added MPs of two size classes (2 and 0.2 mm) to the two soil materials. The added MPs were made of CA in form of microgranules (Merck, Sigma-Aldrich), PP spherules (Merck, Sigma-Aldrich), PP polyfilament (Goodfellow), and polylactic acid (PLA) monofilaments (Nature-Works). For PP and PLA, MPs were created by following a previously developed protocol (Cole, 2016) that is based on aligning fiber-shaped polymers under freezing conditions and cutting them to micrometer-sized particles with a cryo-

genic microtome (cryotome). For the nonfibrous materials, a modified protocol was adopted that involved an initial cutting stage of the materials to obtain ∼1-cm segments followed by multiple steps of sectioning and freezing. This was done by working directly in the cryotome chamber (Microm HM 505 Cryostat microtome) at −20 ˚C in which polymers were fixed and coated on specimen mounts using a freezing solution (Neg 50TM, Richard-Allan Scientific). To evaluate the recovery of the extraction methods, we mixed 30 g of soil from the two matrices (podzol and histosol) to generate a concentration of about three items per gram of soil for each studied polymer and size; this concentration is close to the average concentration (∼2.9 items per gram) in previously studied European soils (Büks & Kaupenjohann, 2020). This mixture resulted in large (∼2-mm) MPs consisting of PLA disks, CA granules, and PP fibers and small (∼0.2-mm) MPs consisting of PLA rectangular foils, CA granules, and PP fragments.

2.2 | MP extraction

To analyze MPs, we applied four extraction protocols and used three replicates for each sample ($n = 24$). The two separation methods forming the base of four extraction protocols were adopted from previous published literature: (a) the oil extraction protocol previously outlined by Crichton et al. (2017), which uses the hydrophobic properties of plastic polymers, and (b) the density separation method (flotation method), which was previously adopted by Imhof et al. (2012), van Cauwenberghe et al. (2013), and Coppock et al. (2017). In short, we explored the efficiency of these extraction methods alone or in combination with an additional sodium hypochlorite treatment. In total, four protocols were used.

Protocol 1 used the canola oil extraction method as described by Crichton et al. (2017) without pretreatment of the sample (Supplemental Figure S1). Protocol 2 used a NaOCl oxidation step added before the Protocol 1 extraction (Supplemental Figure S2). Here, samples were pretreated for organic carbon removal using 1 M NaOCl, with a soil/solution ratio 1:2 for mineral soil samples (podzol) and 1:5 for organic soils (histosol). The treated samples were rinsed with 500-ml ultra-pure water to wash salt from the MP surfaces. We followed a previously suggested method for organic matter removal (Kaiser & Guggenberger, 2003), with the exception that soils were placed in 300-ml Erlenmeyer flasks (500-ml for peat samples), and the supernatant was gently poured into beakers and stored before MP extraction. After the destruction of the organic matter, the supernatant was left to settle overnight. Thereafter, the supernatant was decanted directly into a glass filter funnel equipped with Whatman glass microfiber filter GF/A, and the matrix residues deposited at the bottom of

the beaker were washed back into the Erlenmeyer flasks. The matrices were handled as in Protocol 1. For Protocol 3, we adopted the flotation extraction protocol created by Coppock et al. (2017) but used metal tubes rather than plastic material for the sediment-microplastic isolation (SMI) unit (Supplemental Figure S3). A SMI unit made of metal was judged preferable because it removed eventual contamination of MPs due to polyvinyl chloride scratching and/or chemical degradation. In short, this protocol is based on transfer of the soil sample into the SMI unit, where a solution of sodium hexametaphosphate (NaHMP) with a density of 1.6 g ml^{-1} was used for gravimetrical separation of soil and plastic. The solution was vacuum filtered through a Whatman glass microfiber filter GF/A prior to use. Soils and solutions were manually shaken for 5 min and left to settle for 2 h on a sieve shaker plate so the trapped MPs in the matrix could move into the NaHMP media.

After settling, the valve was closed, and the supernatant in the headspace was vacuum filtered through a 50-µm stainless steel mesh to remove clay and silt residues. The solution media were retained and filtered for further use. The pipe headspace was rinsed meticulously with 500-ml ultrapure water to collect remaining particles. The stainless steel filter was then backwashed onto a Whatman glass microfiber filter GF/A and into petri dishes, where the microplastics were eventually identified.

For Protocol 4, we added a pretreatment step with NaOCl before applying Protocol 3 (Supplemental Figure S4). The supernatant derived from the oxidation step was poured into the stainless steel filter and rinsed with 500-ml ultrapure water to wash salt residues off the MP surfaces. After the vacuum flask was replaced with a clean one, we followed the same procedure as Protocol 3.

2.3 | Effect of NaOCl pretreatment on MP

The ability of NaOCl to deform MP morphologies was investigated by exposing one PLA foil, PP fragments, PP fiber, and CA granules to 5 ml of this oxidant and comparing polymer surfaces of these particles before and after oxidation. We noted complete CA degradation following exposure and repeated the experiment with this polymer using a mass of 1 mg instead. Polymer particles were imaged using a ZEISS EVO LS15 scanning electron microscope equipped with a LaB6 crystal source and scattered electron (model VPSEG4) and back-scattered electron (HDBSD) detectors. Imaging was performed at 5 kV and 200 pA.

The effect of NaOCl on plastic polymers was further investigated with FTIR for spectral recognition XPS for elemental composition changes.

Polyethylene terephthalate from commercially available plastic bottles and low-density polyethylene (LDPE) from packaging foil were manually cut using scissors into 2-mm pieces, and 0.5 g of each microplastic (PP, PLA, CA, PET, and LDPE) were subjected to the same NaOCl treatments as the soil samples. Net changes in weight following the treatments were measured using a calibrated balance (AT261, Mettler Toledo). X-ray photoelectron spectroscopy was used only on the nontreated and treated CA and PET because these polymers were the only ones with a straight surfaces that could be mounted onto the aluminum stub during the electron microscope analysis without any chemical pretreatment. X-ray photoelectron spectroscopy spectra were collected with an electron spectrometer (Axis Ultra DLD, Kratos Analytical Ltd) using monochromatic Al Kα source operated at 150 W. An analyzer pass energy of 20 eV for individual photoelectron line acquisition and a pass energy of 160 eV for wide spectra acquisition were used. The surface potential was stabilized by the spectrometer charge neutralization system. The binding energy scale was referenced to the C 1s line of aliphatic carbon set at 285.0 eV. Spectra processing was accomplished with the Kratos software (Kratos Analytical Ltd).

2.4 | MP identification

Filters obtained from the extractions were investigated under a stereo microscope (Leica) for visual recognition and counting of the spiked microplastics. Validation of visual recognition was conducted using the vibrational spectra of the samples by FTIR (Bruker Vertex 70/v equipped with a DLaTGS detector) using an attenuated total reflectance accessory (Golden Gate, single-bounce, Specac). Spectra were collected in the 60-to-4,000-cm^{-1} range at a resolution of 4.0 cm^{-1} and at a forward/reverse scanning rate of 10 kHz. Each spectrum was the average of 100 scans and was baseline corrected and normalized to the highest peak intensity to facilitate comparison.

2.5 | Statistics

Effects on MP recovery as a function of extraction method, soil type, polymer type, and polymer size were assessed using a generalized linear model where each sample was analyzed using three replicates. Given that recovery represented proportion data, we chose a binomial error structure on the generalized linear model. Data of recovery from individual data frames (soil subsamples) were averaged for each sample to avoid pseudo-replication. When comparing recoveries between extraction methods (Protocols 1 and 3 vs. Protocols 2 and 4), we used a one-way ANOVA (F-test). Statistical analysis was conducted using RGui (version 3.4.3, 2017, R Core Team).
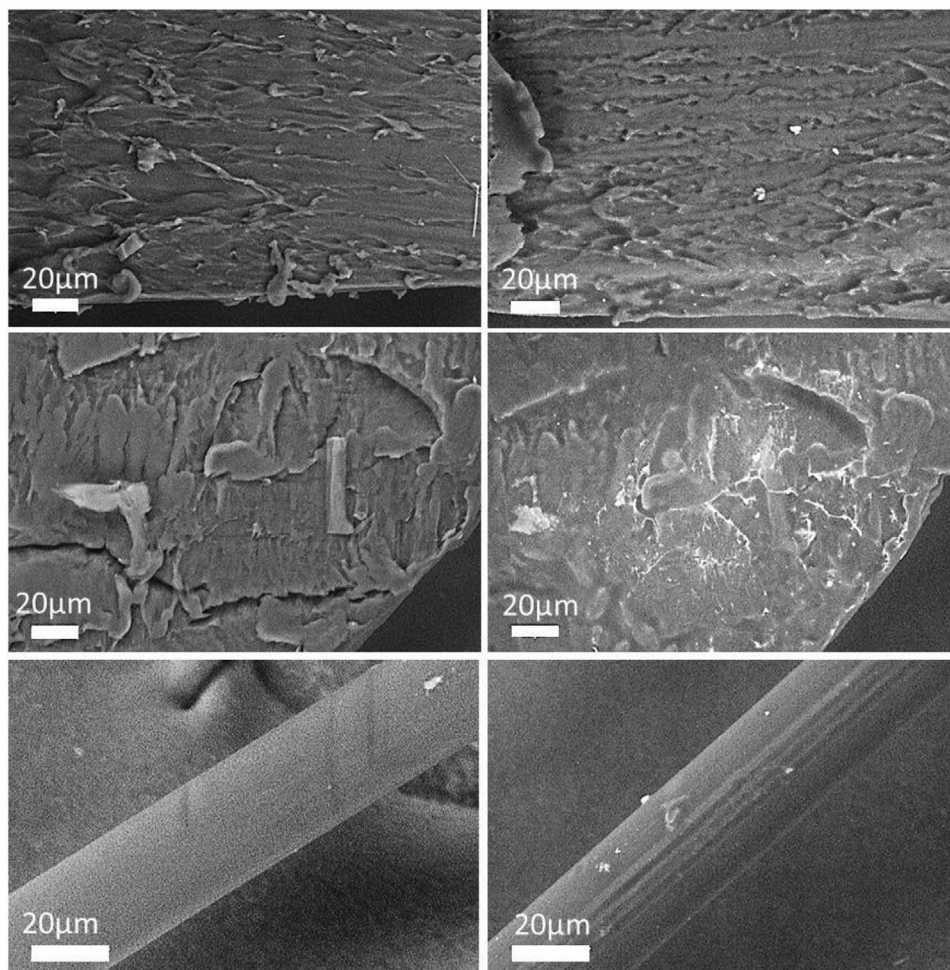
FIGURE 1 Scanning electron microscope images of microplastics before (left) and after (right) NaOCl treatment. Upper panels show surfaces of a polylactic acid particle, middle panels show surfaces of a polypropylene (PP) particle, and lower panels show surfaces of a PP fibers

3 | RESULTS

3.1 | Effect of NaOCl on MPs properties

The results of scanning electron microscopy measurements revealed that NaOCl slightly deformed the borders of PLA and affected the surfaces of PP through peeling and cracking but had little to no effect on the overall morphology (Figure 1). However, NaOCl caused substantial degradation of CA polymers (Figure 2), making it fragile and easy to crack. Substantial CA degradation by NaOCl was further indicated by a 45% weight loss of this polymer after treatment, whereas other polymers experienced a slight gain in mass (4% for PLA, 6% for PP, 5% for LDPE, and 3% for PET).

The XPS analysis revealed that the observed weight gains following our NaOCl treatment co-occurred with formations of new C–Cl bonds on polymer surfaces (Figure 3a–d). Here, the formation of C–Cl bonds at the polymer surface was seen as a Cl 2p line at 200 eV in survey spectra (Figure 3c,d) with a binding energy of Cl 2p 3/2 at 200.0 eV (high-resolution Cl

2p spectra are not shown). However, the atomic concentration of Cl (0.6 atomic %) was close to the detection limit for the CA surface but increased by up to 7% on the PET surface, which was well above the detection limit (0.1 atomic %). For the PET surface, the NaOCl treatment also showed signs of oxidation and formation of additional C–OH bonds, seen as an increase in the C–OH/COOH peak ratio (286.3 eV/288.7 eV) from 1 to 1.5. Chemical alteration of the polymer surfaces due to the NaOCl treatment, however, did not affect their overall FTIR spectra, which remained recognizable after treatment (Figure 4). Only the spectra of the LDPE plastic showed some minor changes following the NaOCl treatment, with two new bands corresponding to C–Cl bonds at 610 and 660 cm^{-1}.

3.2 | MP recovery

Difficulties separating SOM and polymers during the extraction steps in combination with SOM covering filters resulted in 0% recovery of MPs from the histosol (high SOM)
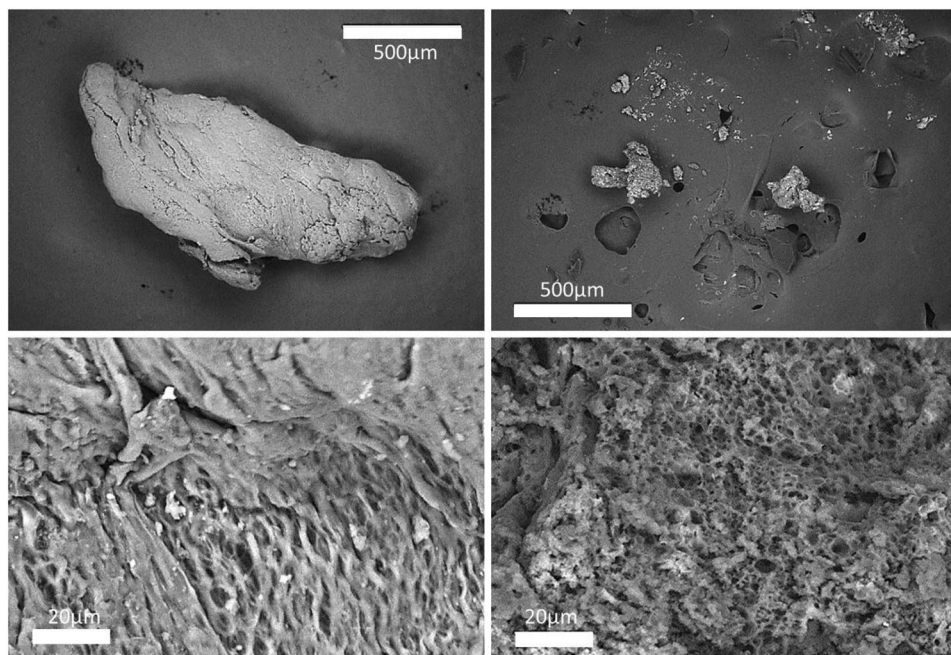
FIGURE 2 Scanning electron microscope images of cellulose acetate plastic particle before (upper left panel) and after treatment with NaOCl (upper right panel). A further magnification of the same granules is shown in the lower panels (left, untreated; right, treatment with NaOCl)

samples without a pretreatment step with NaOCl (Protocols 1 and 3). In other words, in samples with high SOM content (48%), the hydrophobic- or density-based separation methods were unable to recover MPs if NaOCl was not applied (Figure 5). Here, the MP recovery from this organic-rich matrix increased significantly ($\chi^2 = 66$; df $= 1$; $p < .0001$) to 37% with NaOCl. This increase was calculated by comparing the pooled data of Protocols 1 and 3 with Protocols 2 and 4. However, for the podzol (mineral-rich) samples, the proportion of recovered MPs decreased ($\chi^2 = 25$; df $= 1$; $p < .0001$) from 78 to 50% after NaOCl pretreatment.

In addition to matrix type (podzol vs. histosol), other factors (e.g., polymer type and polymer size) influenced MP recovery. Intriguing interaction effects between sample type and polymer properties were evident when comparing recoveries derived using the most promising protocols (i.e., Protocols 2 and 4; Figure 6a–c). First, an interaction effect between soil type and polymer type was observed ($F = 13$; $p < .0001$). This effect was driven by higher recoveries for PP and PLA in the low-SOM-content matrix (podzol) but a contrasting higher recovery of the CA polymer in the SOM-rich (histosol) matrix (Figure 6a). Cellulose triacetate was the only polymer that showed strong mass loss in response to NaOCl. In addition, we detected an interaction effect between applied extraction method and size of the MPs ($F = 5$; $p < .05$). Here, Protocol 4 had a general higher recovery but seemed to be more selective for larger MPs than Protocol 2 (i.e., the latter protocol showed no apparent preference for MPs depending on their size) (Figure 6b). However, we observed a significant interaction effect between MP size and polymer type ($F = 6$; $p < .005$):

compared with larger MPs, smaller CA had a higher recovery, and PP and PLA had a lower recovery (Figure 6c).

4 | DISCUSSION

Our main hypothesis stated that NaOCl could be used to improve recovery of MPs from organic-rich soil samples without large negative effects on polymer chemistry, and polymer morphologies seem valid for most of the studied plastics. When applied to histosol samples, the previous developed hydrophobic-based (Crichton et al., 2017) and density-based (Coppock et al., 2017) MP extraction methods did not detect MPs without an initial pretreatment with NaOCl removing organic matter. That is, we detected MPs only in mineral soil samples with these previously outlined methods. Hence, pretreatment with NaOCl was crucial for recovering MPs in the samples containing >40% SOM. In other words, this reagent showed promise for studies aiming to detect MPs in soil because it not only can remove SOM but also can preserve MP morphology and FTIR spectral signatures of commonly used plastic types (PLA, PP, LDPE, and PET) as well as preserve minerals (Mikutta et al., 2005).

Despite these promising results, our NaOCl pretreatment did have some negative effects. For example, the CA particles, unlike the other studied polymers, became substantially degraded during the oxidation step. This was evident from the observed morphological changes and mass loss during our treatment. However, XPS analysis revealed that the CA polymer maintained its characteristic COOH content after
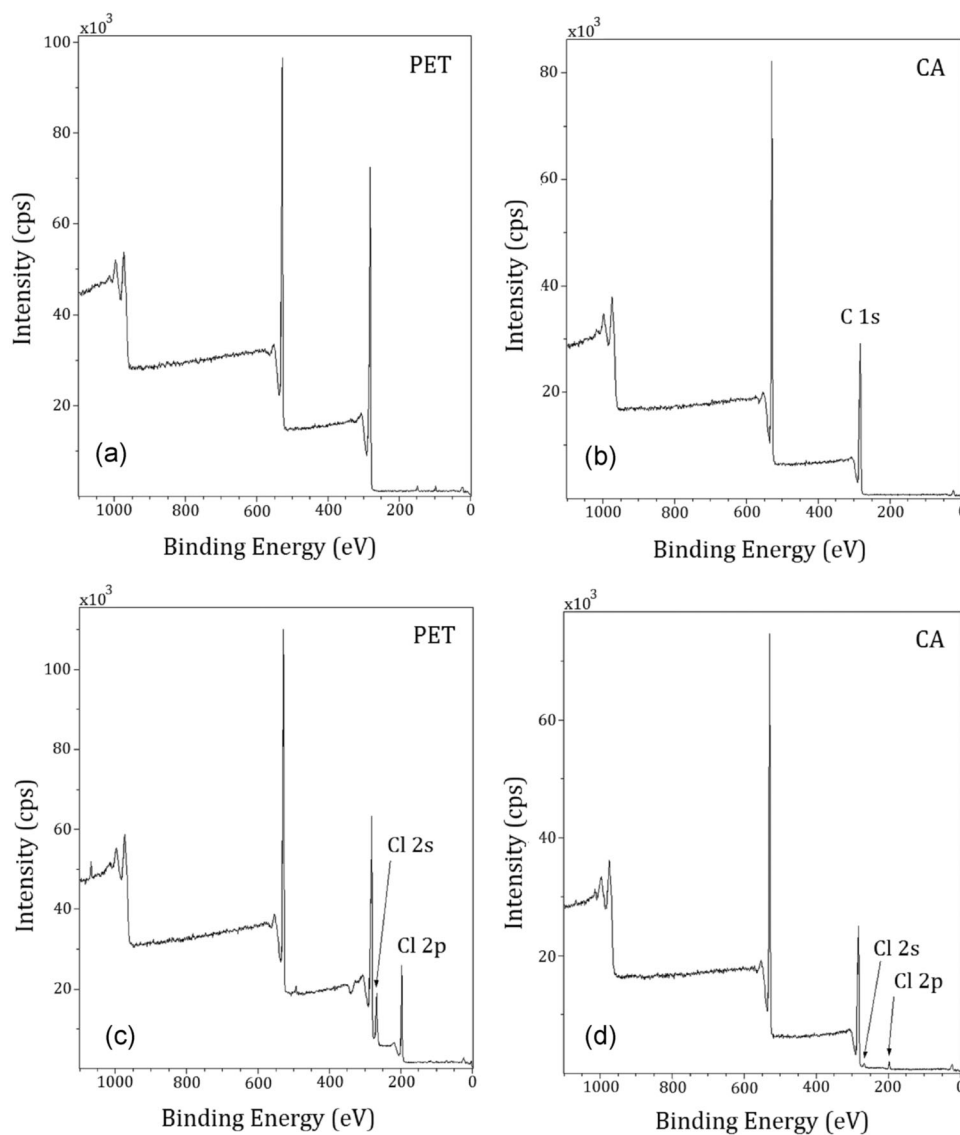
FIGURE 3 Example of wide X-ray photoelectron spectroscopy spectra. Upper panels show polyethylene terephthalate (PET) and cellulose acetate (CA) polymers before treatment with NaOCl. Lower panels show the PET and CA polymer after treatment with NaOCl. Peaks in the spectra at 200 eV indicating chlorination of the polymer surface are indicated with black arrows

exposure to NaOCl, suggesting that qualitative detection of this plastic can still be made after SOM removal with this oxidant. Implicit in this finding is that NaOCl treatment can allow chemical analysis of CA polymers (e.g., GS-MS measurements, FTIR, etc.) but hamper techniques where loss of particle size and mass will lower the success of the detection. In addition to substantial degradation of CA polymers, NaOCl preserved the main properties of all the other studied polymers. Here, the NaOCl treatment caused only minor degradation of the edges and discoloration of PLA polymers. Indeed, discoloration may affect methods based on visual identification of PLA polymers but not those based on spectroscopic methods. Other reagents (e.g., sodium hydroxide and potassium hydroxide [Dehaut et al., 2016; Hurley et al., 2018] and

hydrochloric acid, nitric acid, and H_2O_2 [Cole et al., 2014; Dehaut et al., 2016; Nuelle et al., 2014; Wang et al., 2018] have also been shown to degrade or discolor (bleach) polymers. Bleaching of polymers by H_2O_2 can hamper visual detection of plastics (Nuelle et al., 2014), but discoloration by this reagent does not have a strong effect on the detection rates (Hurley et al., 2018; Radford et al., 2021). As with the effect reported for H_2O_2, we did not find that discoloration from NaOCl-affected polymer detection. However, H_2O_2 is highly aggressive toward mineral constituents, but these minerals remain unaffected by NaOCl at pH <9.5 (Mikutta et al., 2005). Therefore, replacing H_2O_2 with NaOCl would offer an opportunity to combine MP analysis with mineralogical analysis. Moreover, removal of SOM using NaOCl does not
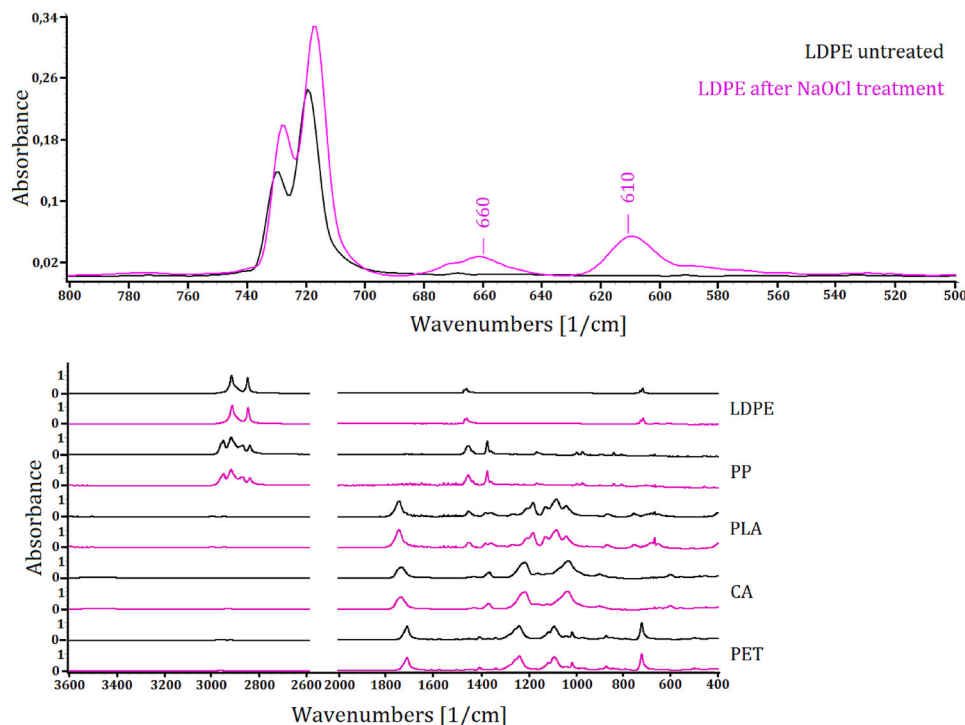
FIGURE 4 Fourier transform near infrared spectrogram of the studied plastic polymers before pretreatment with NaOCl (black line) and after treatment with NaOCl (pink line). Upper panel shows low-density polyethylene (LDPE) plastic particle with a spectrum indicating weak alterations (i.e., halogenation) at lower wave numbers due to the NaOCl treatment. Although other vibrational bands remain, they are largely unchanged by the NaOCl treatment. CA, cellulose acetate; PET, polyethylene terephthalate; PLA, polylactic acid; PP, polypropylene

include a heating step as is typical for H_2O_2 digestions, which may be a practical advantage.

Although it is clear that NaOCl did not considerably alter the FTIR spectral signatures of PLA, PP, LDPE, and PET polymers, some changes in the surface chemical composition were revealed by XPS. Here, the Cl 2p line with binding energy of Cl 2p 3/2 at 200.0 eV (attributed to the bond between organic chlorine and carbon, $C–Cl_2$) appears in the spectrum of the NaOCl-treated polymers. Hence, the slight increase in mass weight after treatment is most likely explained by the chlorination of the polymer surfaces. This interpretation was supported further by FTIR spectroscopy with the appearance of the 660 cm^{-1} band in the treated-LDPE polymer, which is a strong indication of a high degree of chlorination (Nakagawa & Yamada, 1972). For the other polymers, FTIR bands largely overlapped with that derived from C–Cl sites, which made it harder to detect chlorination of their surfaces. As suggested by the XPS analysis of PET and CA, different polymers have different degrees of halogenation, probably due to the number of sites available in the polymer structure for C–Cl bond formation or to a different concentration of antioxidants present as additives.

A recent comparison of extraction methods concluded that hydrophobicity-driven (oil) extraction of MP is preferable in comparison to density-dependent extractions, but this method might be more sensitive to high organic matter content (Radford et al., 2021). In our study, recoveries were sig-

nificantly higher for the density-dependent separation methods in comparison to the hydrophobicity-driven extraction. Considering that our results seem to contrast with the conclusion made by Radford et al. (2021), the generality of our finding remains uncertain. It seems rational to expect that the observed NaOCl-induced alteration of polymer surfaces (chlorination and additional C–OH bond formation) might have contributed to a reduced efficiency for the canola oil extraction. Sodium hypochlorite–induced chlorination and polarization of the polymer surface can reduce hydrophobic properties (Iguerb et al., 2006), which the oil-based separation method uses to separate the soil matrix from the MPs. Note that the XPS revealed formation of additional C–OH bonds at the polymer surface due to oxidation of PET hydrocarbon chain and/or carboxylic groups; therefore, oxidation per se might alter the hydrophobicity of MPs. However, recoveries were generally higher for the density-based separation method (Protocol 3) than for the oil-based extraction (Protocol 1) even for the untreated podzol samples. This difference suggests that NaOCl-induced alterations of the surface properties were unlikely the only factor behind the lower recoveries for the oil-based extraction. Although interference from SOM generated a lower recovery for the resistant polymers, a humus-rich matrix reduced oxidative stress on the polymers and increased preservation of the most easily degradable CA particles. Here, reduced oxidative stress in the presence of SOM likely explained the higher recovery of CA
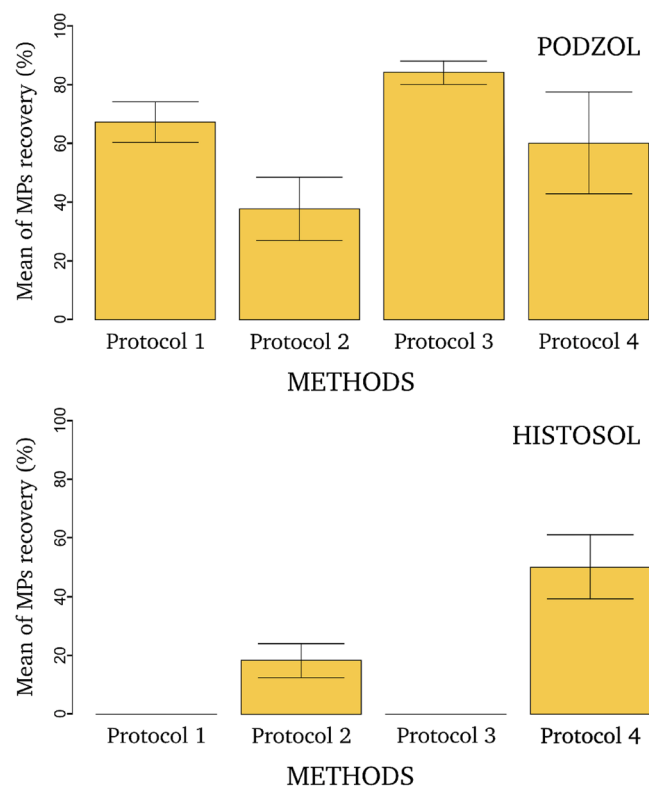
FIGURE 5 Average recovery of microplastics (MPs) as a function of used protocol and soil type. Upper panel shows the result of the four tested protocols when applied to the mineral soil (podzol), and the lower panel shows the result when applied to the soil organic matter–rich (histosol) sample. Error bars indicate standard error ($n = 6$)

MPs in the organic matter–rich soil compared with the podzol samples.

To our knowledge, our study is the first to statistically assess how MP recoveries are affected by polymer properties (size and type), extraction techniques (separation based on density or hydrophobicity), and the soil matrix itself. Our experiments show that smaller MPs have lower recoveries than larger MPs, which is logical considering that the probability of being visibly detected increases with size. Here, the higher recovery of the smaller CA polymer (the only polymer showing this counterintuitive size trend) was caused by the degradation of larger-sized MPs, so they were placed into the small size category after the NaOCl treatment. However, the recoveries depended not only on the size of the MPs but also on the separation method, polymer type, and soil matrix. Compared with the polymer type and separation methods, differences in MP sizes played a minor role in observed recoveries in our experiments. Lu et al. (2021) recently called for standardizing analytical approaches for MPs to facilitate comparison between studies. Our findings support this view, but interaction effects between polymer type, size, soil type, and extraction methods also underscore the difficulties involved when finding an approach that works for all soils and all MPs. Future work on the fate of MPs in soils may still need to adjust their analytical approach depending on MP type and soil matrix. Nevertheless, we note that Protocol 4 was able to detect MPs in five different urban soil types (Supplemental Table S1), indicating its potential use for qualitative studies of MPs in urban environments.
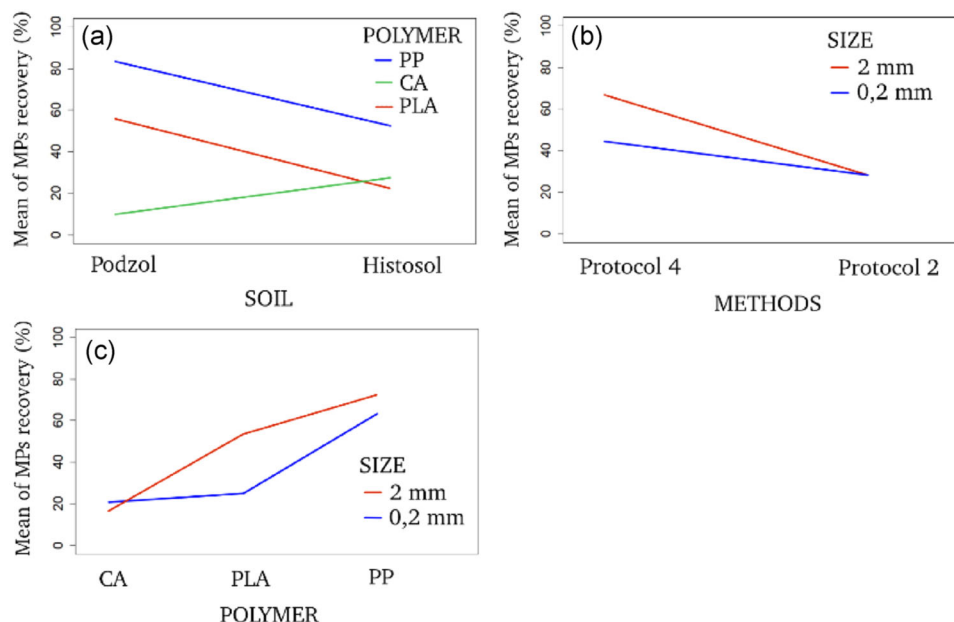

FIGURE 6 Plots showing how the microplastic (MP) recoveries depend on (a) polymer type, (b) extraction methods, and (c) polymer size. These figures highlight significant interaction effects (i.e., effects where the recoveries [dependent variable] show different responses depending on the independent variables [polymer type, extraction method, and polymer size]). The size of the particles is shown in the figure, and the statistical information is provided in the Results. CA, cellulose acetate; PLA, polylactic acid; PP, polypropylene

5 | CONCLUSION

For high-SOM soils (e.g., >40%), pretreatment with NaOCl offers an effective room-temperature approach that reduces interference from humus during MP separation while allowing assessment of how the particles interact with poorly crystalline minerals. Chlorination of polymer surfaces following NaOCl reduced plastic hydrophobicity, suggesting that density-dependent (flotation) separation methods are likely preferable when this reagent is used for MP analysis. Nevertheless, recovery of MPs depends not only on extraction methods but also on the size of the plastic, polymer properties, and SOM content. Clearly, developing a universal analytical approach that provides quantitative estimates of MP abundance for all plastic types and environmental conditions is challenging.

AUTHOR CONTRIBUTIONS
Anna Bottone: Conceptualization; Data curation; Formal analysis; Investigation; Methodology. Jean-Francois Boily: Data curation; Methodology. Audrey Shchukarev: Data curation. Patrik L. Andersson: Methodology; Writing – review & editing. Jonatan Klaminder: Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Writing – original draft; Writing – review & editing.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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