Dear referees,

thank you very much for your engagement in the re-review process and the nice and helpful advices. In the following you can find our modifications. Replies to the your comments are color marked with [numbers], further rephrasings (without changing the content) are only color marked.

Best regards,
Dr. Frederick Büks

Answers to Referee #1

[1] The statement that plastics age and therefore that fate and process studies in soils should ideally be done with aged plastics rather than juvenile (pristine/non-aged) plastics, seemingly the main point the paper argues for, seems obvious, raising the question if this point needs such a paper contribution.

→ There are a lot of studies that still use pristine plastic and neglect the influence of aged surfaces. However, this is not the focus of the present manuscript, but the additional contribution of underground weathering. The biogeochemical aging of MP surfaces in soil seems obvious due to the the transformative nature of soils, but there are some inconsistent indications that urge to study the extent of this effect: On the one hand, there is some work claiming extensive stability of plastic in soils and also works that show stability of MP in face of some stressors (e.g. Oberbeckmann and Labrenz, 2020; Büks et al., 2021), but on the other hand there are indications for susceptibilities discussed in this work. We hope to address this contradiction within the first part of chapter 3.

[2] While the manuscript addresses some of the possible weathering processes (in a rather rudimentary manner), statements or hypotheses for which processes in soils such aging may become relevant are not made. (...)

Line 59: this is a rather crude description of photochemistry. It's not that photons "hit weak bonds" thereby breaking them. In direct photolysis, photons are absorbed, an excited state is formed which can result in bond cleavage. In indirect photolysis, photochemically produced reactive intermediates form that can attack chemical bonds.

→ Since there is extensive literature on photooxidative weathering, the rudimentary description in our manuscript is now strongly condensed: "On a microscopic scale, the surfaces of pristine plastic items are normally smooth with nearly no surface charge (e.g. Fotopoulou and Karapanagioti, 2012; Fotopoulou and Karapanagioti, 2015). Exposed to sunlight, a depletion of UV absorbers and HALS (hindered amine light stabilizers) leads to enhanced photoxidation (e.g. Kokott, 1989; Pickett, 2018). From the point of view of the macroscopic observer, the plastic becomes less hydrophobic, stiff and more prone to fragmentation by wind and water erosion."

We further specified soil processes, that might be affected by changing surface properties of MP: soil structure, edaphon health, transport of plastic and soluble substances (see first paragraph of chapter 3).

[3] Line 18: Any plastic particle entering soils will undergo additional changes on its surface. It seems that this is not a mere possibility but, in fact, a given!

→ We specified, that we don't know how extensive these changes are: "When plastic particles then enter the soil environment, further aging factors appear with yet unknown efficacy."

[4] Line 19: Decay with enzymes seems limited to specific plastics whereas conventional plastics are resistant to enzymatic attack. This needs to be expressed more clearly considering recent studies claiming that conventional plastics are enzymatically degradable. This is simply not the case.

→ Wei and Zimmermann (2017) and others reviewed experiments, that showed the degrading effect of enzymes on conventional and biodegradable plastics (see line 121-131). Only the magnitude of the effect in complex systems is unclear. We therefor added "(… with both conventional and biodegradable plastics), …" to line 19.

[5] Biotic and abiotic acids? What is meant here? Grammatically incorrect it seems.

→ Right you are. We changed the sentence: "…, contact with biotic and abiotic acids, oxidants as well as uptake by the soil fauna that causes physical fragmentation." Biotic acids include root exudates, abiotic acids are e.g. carbonic acids or nitric acid. In both cases, it cannot be ruled out that they lead to long-term weathering of embrittled plastic (although we know, that e.g. laboratory equipment is very stable in face of most of the acids).

[6] Line 20: it clearly is desirable to work with plastic objects that mimic those in nature. But for which types of experiments is this relevant? For instance, persistence of the particles unlikely is changed by these modifications. Transport characteristics also not for larger particles (but maybe for nano-sized particles). (…) The reviewer would have appreciated a bit more guidance as to which processes are affected rather than a relatively obvious statement that pristine plastic does not equal aged / weathered plastics.

→ We added "Such transformation of surfaces is assumed to affect soil aggregation processes, soil faunal health and the transport of plastic colloids and adsorbed solubles." to line 21 (see also [2]).

[7] Is it possible that all plastic "looks alike" in soils if chemically different materials obtain the same "coating" (ecocorona)?

→ This is really a very interesting point. We included it by changing lines 113-119 as follows: "Recent studies on soil ecosystems have also demonstrated that MP surfaces of different origin are covered with microbial communities. This might hypothetically cause a masking of plastic surface characteristics by the biofilm matrix. The composition of surface MP communities is very different from that of the soil matrix (Chai et al., 2020; Zhang et al., 2019). The altered soil microbial community is thereby not only determined by the physiochemical properties of the surrounding soil, but also by the type of plastic and its additives (Chai et al., 2020; Ng et al., 2020; Wang et al., 2020; Wiedner and Polifka, 2020; Yan et al., 2020; Yi et al., 2020). This might lead to a physiochemical behavior of plastic particles, that differs not because of the plastic type, but because of its biofilm cover."

[8] Line 24: What is meant with "young"? Can plastic be young? The authors mean pristine? Non-aged?

→ Thank you. We now use "pristine" in the sense of "not aged" instead of other terms throughout the manuscript.

[9] Line 41: Sure. But these are studies that used plastics in the mass% range? How realistic is this? And is this not a rather trivial finding that a soil with, lets say 10 mass % of fine ground plastic, is no longer behaving like a soil without 10% of plastic? A soil with 10% more sand or clay (or anything) will change its properties as well.

→ We strongly agree, that – especially in experiments with massive addition of MP – a clear distinction between adverse effects caused by the MP itself and those caused by changing physicochemical conditions is necessary. However, from our point of view, this is beyond this forum article.

[10] Line 51: it is true that plastics exposed to the elements undergoes weathering. However, many of the plastics contain additives to prevent chemical transformation. UV stabilizers, pigments, antioxidants … . Is it possible that for many plastics photochemical weathering is small because of these additives? Also, given that plastics differ not only in polymeric composition but also types and concentrations of protective additives, what do the authors suggest? Case-specific weathering of commercial items? Clearly, weathering PE from vendors like Sigma Aldrich or Fisher Scientific, etc would not help if these are, for instance, not photostabilized.

→ We added "depletion of UV absorbers and HALS (hindered amine light stabilizers)" to [2] to clarify, that aging is hindered as long there is intact protection.

[11] Line 54: what is a microscopic perspective?

→ Better: "On a microscopic scale …"

[12] Line 55: "Uniformly structured" seems a simplification: for instance, semicrystalline polymers contain amorphous regions and crystalline lamella which behave very differently.

→ You're right, AFM images clearly show this oversimplification. We deleted "... and uniformly structured …"

[13] Line 58: "photooxidation and indirect photolysis". This is incorrect. Photooxidation is a chemical reaction that can result from both direct and indirect photolysis.

→ See modification in [2].

[14] Line 79: recommend instead of advice

→ Thank you. Done.

[15] Line 83: is gamma irradiation a relevant aging process in the environment?

→ Fortunately not, but used to accelerate aging. To avoid misconceptions: "γ-irradiation treatment".

[16] Line 85: well, any oxidative process would, no? there are many ways of introducing surface oxygen functionality into polymers.

→ Sure, but this is the recent work on this topic.

[17] Line 93: why "dimmed". Not dark?

→ In the first mm of soil there are more dimmed than dark condition. But dark is largely correct. Done.

[18] Line 100: Why is soil fauna a "bioreactor"? Sure, they are "alive" and hence "bioreactors". But most plastics will not be affected chemically when passing through these organisms. If humans swallow stones, would it be correct to say they are "bioreactors"? The reviewer would argue that the stone comes out as it went in (except for some coating). Bioreactors seems to invoke the false impression that the plastic is significantly processed.

→ The intestinal tract of the soil fauna provides an environment with conditions enhancing the activity of the soil microbiome. Here, MP is covered with biofilms and occluded into casts. These fundamental processes justify this terminology, although the efficacy of plastic aging in the GIT is unknown.

[19] Line 105-106: Does this make PS and PE non-persistent? By no means.
→ If additives are depleted, the polymer is more susceptible to the environment. And if then the polymer is degraded, it is clearly a sign of non-persistence. See modification [33].

[20] Line 106: First, there is relative humidity: a chemical reaction involving water does not need "pure liquid". Second, which reaction can water perform on conventional plastics? Hydrolysis reactions? No.
→ Thank you. We deleted "… and is then not an important factor of weathering (Pickett, 2018), …" In the soil, liquid water plays a major role for nearly all aging processes in question.

[21] Line 114-115: Any surface in a soil will lead to enrichment of specific microorganisms. This per se is not a surprising finding. A leaf added to soil will have a surface microbial community that differs from that in the soil.
Sure. From our point of view, there is no reason to skip this point within the discussion of surface alteration and masking (see [7]).

[22] Line 134-137: But these polymers remain recalcitrant even if some enzymes (in lab incubations?) oxidize some parts of these polymers.
→ Oxidation of parts of the plastic surface causes surface alteration. If the oxidation is going on, there is an increasing alteration, and this leads to long-term degradation. But of cause, we have to mention the laboratory character of the experiments: "Given a poor biodegradability of polymers with C-C backbones and no hydrolysable functional groups such as juvenile PE, PP, PS and PVC, laboratory experiments showed an unexpected
degradation of PE by a bacterial alkan hydroxylase (Yoon et al., 2012), and, beyond this, the specific targeting of PET with a bacterial PETase (Yoshida et al., 2016)."

[23] Line 148: soil pH affects the surfaces? How so? Do they contain pH-sensitive acid/base groups?
→ Thank you, the sentence was mistakable. Not variable charge, but "soil born acids and oxidants".

Answers to Referee #2

[24] It still has lengthy paragraphs of review character that should be shortened to the information relevant to substantiate the authors' view point.
→ Thank you, we condensed the descriptions of photooxidative aging (see [2]) and artificial weathering (line 70-91).

[25] The main title and the section titles do not reflect the article's view point character and the authors' intent to call for a new methodologic approach in the microplastics research community.
Title should reflect the intention of the paper: an appeal to where research should be directed.
→ We changed the title to "What comes after the sun? – On the integration of soil biogeochemical pre-weathering into microplastic experiments". We also merged the two weathering chapters to "Underground weathering – a second phase of aging?" and renamed the conclusion to "Pre-weathering under soil conditions: A methodology for future approaches?"

[26] Consider to use the more common term "pristine" instead of "juvenile"
→ Done (see [8]).

[27] Some expressions/wordings are a little clunky in parts and quite a few grammar errors and typos occur throughout. I'd recommend a revision by a native speaker to ensure the language is easy to read and inambiguous, and also to shorten the sentences.

→ Thank you very much for that advice.

[28] Line 13: replace "MP fraction" with "MP size fraction"

→ Done.

[29] Line 24: why this title? Rather, a point should be made that studies so far have widely ne-glected the dimension of time of plastic exposure to environmental factors.

→ We changed the section title to "Did we neglect biogeochemical aging factors?"

[30] Line 53 ff: instead of the following two sections divided into photooxidative and "biogeo-chemical" weathering, I would recommend to craft this into one section. In such, the authors can get to the point, using less words, which types of weathering relevant for soil environments have been considered in past studies and which have not.

→ Done (see [25]).

[31] That said, mechanical effects that have been assigned solely to the underground part (that is how I in-terpret "dimmed world of soil fauna…") might happen as well at the surface, e.g. during abrasion with soil particles caused by wind/water erosion.

→ See [2].

[32] Line 72: Please provide a link to this initiative and translate for the international reader-ship

→ Done: "… Plastic in the Environment", https://www.bmbf-plastik.de/en, ...).

[33] Line 105: I am not sure I understand the meaning of "to an eminent degree beyond the proportion of additives"

→ "There are also indications that the mealworm microbiome is able to degrade not only additives, but also PE and PS polymers."

[34] Lines 137 ff: I am missing the argument that plastic is unlikely to serve as a major sub-strate for microbes due to their large molecular size, high chemical stability, and low bio-availability (Oberbeckmann and Labrenz 2020)

→ We added "Although plastic is unlikely to serve as a major substrate for microbes due to its large molecular size, high chemical stability, and low bioavailability (Oberbeckmann and Labrenz 2020), there is indication that a biofilm causes the alteration of MP surfaces." to line 118.

[35] Table 1: Why put (?) if there are references?

→ Sorry, leftover. Deleted.

[36] Line 156: replace "mechanical treatment through biota" with "biotic effects".

→ Done.

[37] Line 163 ff: This title is not very meaningful, and also this section has too much "review-character". For a view point paper, the authors should focus more on what they think is needed for future studies, but not putting too much detail into the techniques of weathering simulation that have been applied in the past.

→ We added the focus on future practice (see [1]), but prefer to keep the description of past techniques, since there is no work that collects those information.

[38] 1. If this was the case, would it not have tremendous implications on the quantification methods currently used? The stresses are presumably much stronger in the extraction set-ting compared to a soil in situ. In fact, can some of the separation methods be considered accelerated weathering (e.g. breaking down soil aggregates in a mortar, using acidic rea-gents, enzymatic digestion)? Or can we assume that these effects are negligible in situ, if microplastic particles resist the harsher conditions in the lab?

→ That is a very interesting point. From our point of view, cavitational stress via ultrasound and e.g. gnawing are not the same type mechanical stress and, thus, stability of MP in face of ultrasound as shown in Büks et al. (2021) is not representative for feeding on MP. A discussion of that point, I think, would exceed the format of the forum article.

[39] 2. However, in the case that microplastics are altered to a significant degree once they enter below the soil surface, why should weathering simulation methods be established before we even know if these processes alter microplastics behavior and impacts, e.g. on organisms or soil structure? Should the community not prioritize on effect studies that compare pristine with weathered particles regarding their effects on soil biota, accumulation, transport, etc.? What about an alternative approach of studying the effects in soils that have been contaminated under real conditions in field studies of soils with high “legacy” microplastic contamination and controls with low levels (e.g., in long term sewage sludge or compost trials)?

→ Thank you. We tried to address this step-by-step approach throughout the manuscript.

[25]What comes after the sun? – On the integration of soil biogeochemical pre-weathering into microplastic experiments

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Abstract. Recent studies have been engaged in estimating the adverse effects of microplastic (MP) on soil quality parameters. Mass concentrations of MP as found in highly contaminated soils have been shown to weaken the soil structure, and parts of the edaphon are adversely affected by mainly the < 100 µm MP [28]size fraction. However, the vast majority of these studies used [8]pristine particles, which have surface characteristics different from that of environmental MP. Exposed to UV radiation, plastic undergoes photochemical weathering with embrittlement and the formation of surface charge leading to an alteration of physiochemical behavior. When plastic particles then enter the soil environment, [3]further aging factors appear with yet unknown efficacy. This little explored soil biogeochemical phase includes biofilm cover, decay with enzymes (as shown in laboratory experiments [4]with both conventional and biodegradable plastics), [5]contact with biotic and abiotic acids, oxidants as well as uptake by the soil fauna that causes physical fragmentation. [6]Such transformation of surfaces is assumed to affect soil aggregation processes, soil faunal health and the transport of plastic colloids and adsorbed solubles. This perspective article encourages to consider the weathering history of MP in soil experiments and highlights the need for reproducing the surface characteristics of soil MP to conduct laboratory experiments with close-to-nature results.

[29]Did we neglect biogeochemical aging factors?

Since the mass production of plastic articles of daily use started in the early 1950th (Thompson et al., 2009), a number of processes cause the contamination of ecosystems such as inland and coastal waters, sediments, the open and deep seas, soils and even the atmosphere with MP (e.g. Cole et al., 2011; Woodall et al., 2014; Wu et al., 2018; Büks and Kaupenjohann, 2020; Trainic et al., 2020). The formation of soil MP pools occur through littering and dispersion from landfills, the application of wastewater, contaminated surface water, sewage sludge, composts, digestates, mulching foils, seed and fertilizer coatings, road dust as well as atmospheric deposition (Eerkes-Medrano et al., 2015; Huerta Lwanga et al., 2017a; Weithmann et al., 2018; Corradini et al., 2019; Dierkes et al., 2019; He et al., 2019; Edo et al., 2020; Huang et al., 2020; Bertling et al, 2021; Katsumi et al., 2021; Szewc et al., 2021).

Today, we are faced to a global contamination of soil ecosystems with MP, that averages 1.7 mg kg^{-1} dry soil in agricultures (Büks and Kaupenjohann, 2020), exceeds this value by several orders of magnitude in heavily contaminated soils at road sides and industrial areas (Fuller and Gautam, 2016; Dierkes et al., 2019), and reaches even remote areas (Abbasi et al., 2021). Several laboratory studies showed adverse effects of high MP concentrations on the soil fauna (Büks et al., 2020a) and soil structure (e.g. de Souza Machado et al., 2018; de Souza Machado et al., 2019; Liang et al., 2019; Lozano et al., 2021) and underlined the relevance of especially the small-sized fraction (MP<100 µm) (Büks et al., 2020b).

Although these results are rightly alarming due to the function of soil structure and the edaphon as soil fertility parameters (Bronick and Lal, 2005; Thiele-Bruhn et al., 2012), their informative value is limited by the fact, that the vast majority of experiments used [8]pristine plastic and a short run time that does not allow for further weathering (e.g. de Souza Machado et al., 2018; de Souza Machado et al., 2019; Liang et al., 2019; Büks et al., 2020a; Lozano et al., 2021). For a better matching with of environmental conditions, some studies have used photooxidatively weathered plastic. In soil, however, the bulk of MP is additionally exposed to biogeochemical alteration for years. Its surface characteristics and role within soil ecosystems thereby possibly change compared to solely above ground weathering.

[25]Underground weathering – a second phase of aging?

[2][11]On a microscopic scale, the surfaces of pristine plastic items are normally smooth [12]with nearly no surface charge (e.g. Fotopoulou and Karapanagioti, 2012; Fotopoulou and Karapanagioti, 2015). Exposed to sunlight, [10]depletion of UV absorbers and HALS (hindered amine light stabilizers) leads to enhanced photooxidation (e.g. Kokott, 1989; Pickett, 2018). From the point of view of the macroscopic observer, the plastic becomes less hydrophobic, stiff and more prone to fragmentation [31]by wind and water erosion.

[24]Standardized approaches from materials science are newly used in soil science to reproduce natural photooxidative aging characteristics (BMBF initiative “Plastic in the Environment”, [32]https://www.bmbf-plastik.de/en, e.g. Büks et al., 2021). They [14]recommend xenon arc lamps with borosilicate filters, that adjust the emitted spectrum tighter to the natural UV spectrum (DIN EN ISO 4892-2), or fluorescent UV lamps (DIN EN ISO 4892-3). The performance of these approaches is enhanced by use of modern daylight filters, a steady temperature of 38°C, relative air humidity of 25 to 50 % and regular washing of the sample surfaces by artificial rain (Pickett, 2018). Beside the use of UV, γ-irradiation [15]treatment is reported to imitate the carbonyl stretch in PE samples similar to a long-term UV-B exposition (Johansen et al., 2019). Furthermore, Zhou et al. (2020) could demonstrate that [16]discharged plasma oxidation (DPO) is likewise suitable to increase surface area, crystallinity and carbonyl indices of plastic particles within hours.

However, it is unknown whether these protocols properly reproduce the additional influence of underground aging, which occurs under different physiochemical conditions. When plastic is exposed to the [17]dark world of soil fauna, microorganisms, roots and frequent leaching, the composition of weathering parameters changes significantly (Table 1). The plastic is now faced to new mechanical stresses such as (bio)turbation, largely moist conditions and exposed to a variety of soil biogeochemical processes.

One of these potential aging factors is the diverse and active soil fauna, that has been shown to ingest, digest and excrete plastic particles (Büks et al., 2020). It is an ensemble of small, mobile [18]bioreactors, that incubate soil particles including MP within a habitat of high microbial diversity – their gastrointestinal tract – and distribute them throughout the soil by excretion. A well known example for this multifaced functionality is the earthworm. Some taxa like woodlice, termites, mealworms and earthworms have been additionally found to comminute plastic by gnawing and, hence, actively produce MP (e.g. Lenz et al., 2012; Zhang et al., 2018; Büks et al., 2020a). [19]There are also indications that the mealworm microbiome is able to degrade [33]not only additives, but also PE and PS polymers (e.g. Brandon et al., 2018).

While moisture evaporates quickly on sun-exposed, heated plastic surfaces[20], in soils it is the ubiquitous condition for microbial life, extracellular metabolic processes and the release and transport of chemical agents, that react with the plastic outside the fauna. Microbial colonization and biofilm formation on surfaces of MP particles have been shown in studies on various aquatic ecosystems (e.g. Zettler et al., 2013; McCormick et al., 2014; Oberbeckmann et al., 2015; Dussud et al., 2018; Jiang et al., 2018). [7]Recent studies on soil ecosystems have also demonstrated that MP surfaces of different origin are [21]covered with microbial communities. This could hypothetically cause a masking of plastic surface characteristics by the biofilm matrix. The composition of surface MP communities is very different from that of the soil matrix (Chai et al., 2020; Zhang et al., 2019). The altered soil microbial community is thereby not only determined by the physiochemical properties of the surrounding soil, but also by the type of plastic and its additives (Chai et al., 2020; Ng et al., 2020; Wang et al., 2020; Wiedner and Polifka, 2020; Yan et al., 2020; Yi et al., 2020). This might lead to a physiochemical behavior of plastic particles, that differs not because of the plastic type, but because of its biofilm cover.

[34]Although plastic is unlikely to serve as a major substrate for microbes due to its large molecular size, high chemical stability, and low bioavailability (Oberbeckmann and Labrenz 2020), there is indication that biofilm cover causes the alteration of its chemical properties. Not only a viscous matrix, that protects bacteria against mechanical stress, predators, desiccation and irradiation, biofilm is also an extracellular reaction space that facilitates the concentration and metabolization of nutrients and the recycling of dead cell material (Flemming and Wingender, 2010). For this purpose, manifold extracellular enzymes are produced by the biofilm community to decompose food sources or modify the biofilm matrix in face of e.g. oxygen or nutrient gradients (Flemming and Wingender, 2010). Among these are esterases, proteases and amidases that target on substrates like polysaccharides, proteins, extracellular DNA, lipids and urea, but also allow the (co)metabolization of artificial polymers such as diverse polyesters, ester-based PU and PET in laboratory experiments (Shimao, 2001; Wei and Zimmermann, 2017; Danso et al., 2019).

Given a poor biodegradability of polymers with C-C backbones and no hydrolysable functional groups such as [8]pristine PE, PP, PS and PVC, [22]laboratory experiments showed an unexpected degradation of PE by a bacterial alkan hydroxylase (Yoon et al., 2012), and, beyond this, the specific targeting of PET with a bacterial PETase (Yoshida et al., 2016). In contrast, neither degrading enzymes nor observed biodegradation have been reported in case of PP and PVC (Danso et al., 2019). Unspecific lignin-degrading enzymes such as laccases, manganese peroxidases, hydroquinone peroxidases and lignin peroxidases produced by actinomycetes, other bacteria as well as fungi, have been further shown to depolymerize even plastics such as PE, PS and PA, that are considered recalcitrant (Bhardwaj et al., 2013; Wei and Zimmermann, 2017). Beside the direct proof of enzymatic degradation pathways there are numerous references on the metabolization of (bio)plastic samples by bacterial and fungal strains (e.g. Bhardwaj et al., 2013; Kale et al., 2015; Raziyafathima et al., 2016; Roohi et al., 2017). However, since many studies applied commercial polymers, that have concealed compositions (Danso et al., 2019), there is often poor insight to what degree the measured mass loss is caused by microbial/enzymatic decomposition of the polymer or additives. These findings imply, that biodegradation of plastic surfaces in soil is conceivable.

Table 1: Development of surface characteristics during the three phases of aging ([8]pristine, photooxidative and soil biogeochemical phase). Data of soil biogeochemical weathering are only known from aquatic systems. (?) marks assumptions based on soil biogeochemical processes found in soils. Some references are: [1]Fotopoulou and Karapanagioti (2012), [2]Fotopoulou and Karapanagioti (2015), [3]ter Halle et al. (2017), [4]Dong et al. (2020), [5]Pickett (2018), [6]Andrady et al. (1993).

characteristic	**[8]pristine phase**	**photooxidative phase**	**soil biogeochemical phase[35]**
topography	smooth[1,2,4]	rough[5]	rough[1,2,4]
surface charge, carbonyl index	no[1,2,3,4]	yes[6]	increasing[1,2,3,4]
crystallinity, crosslinks, chain scissions	low[3]	high[5]	increasing[3,4]
biofilm cover	low	low	growing or mature[2,5]
aging factors	no	UV radiation[5] blue/violet spectrum[5] frequent leaching[5] wind/water erosion	enzymes(?) organic acids(?) inorganic acids(?) bases(?) oxidants(?) bioturbation(?) feeding by the edaphon(?) frequent leaching(?) freeze-thaw-cycles(?)

Beside the soil biome, [23]soil born acids, bases and oxidants are expected to directly influence the belowground alteration of plastic surfaces. While – to the best of our knowledge – there has been no systematic examination of the effects of such agents within natural ranges of concentration and time of exposure, the treatment of plastic with highly concentrated reagents caused damaging effects from color leaching and expansion to total dissolution (Enders et al., 2017). However, pre- and post-treatment with oxidants such as H_2O_2 are common parts of the extraction of MP from soil samples with density fractionation (Büks and Kaupenjohann, 2020).

In winter, when the [36]biotic effects are reduced, freeze-thaw-cycles might be an additional factor of fragmentation. Studies on the effect of alternating freezing and thawing on the structure of plastic surfaces are sparse and only focus on composite materials that include non-plastic components (Wang et al., 2007; Adhikary et al., 2009; Zhou et al., 2014). However, water, that has entered cracks of brittle plastic, most likely contributes to its fragmentation through freezing and expansion and, thus, increases the surface area exposed to other aging factors.

[25]Pre-weathering under soil conditions: A methodology for future approaches?

[1]While plastics are considered persistent, the above in-vitro experiments indicate, that degradation in soil is possible. The difference in factors leading to photooxidative and biochemical weathering make it plausible for MP surface characteristics to develop differently in above- and belowground environments. If the physicochemical behavior of the microplastic is significantly affected by this, the effects must be considered in the design of laboratory and field experiments. The focus on surfaces is particularly important in studies on (1) soil aggregation and structure, that strongly depend on biofilm cover and surface charge/polarity of the involved primary particles, (2) adverse effects on the soil fauna, that might be influenced by particle shape and sorption of (in)organic pollutants, (3) interactions with plants and microorganisms as well as (4) the transport of colloidal MP within the soil pore space.

However, there are currently no studies that evaluate the efficacy of specific soil biogeochemical aging mechanisms. Recent work only showed the alteration of plastic surfaces during environmental weathering, indicating that future experiments have to be conducted with pre-weathered instead of [8]pristine MP. It is still an open question, if there is effective soil biogeochemical aging beyond the photooxidative phase or whether the DIN EN ISO 4892-2/3 approach, as applied in recent work, is sufficient to imitate soil weathering conditions in future studies.

Only a few studies have integrated soil biogeochemical factors into pre-weathering approaches of artifical MP so far (Table 2, Büks et al., 2020a), alas fragmentary, heterogeneous and often directly applied to [8]pristine plastic: Tsunoda et al. (2010) heated plastic items within a water bath at 90 °C for 3 weeks and abraded the surface prior to feeding experiments with termites. This treatment was aimed to make the surface more accessible for gnawing and might also extract soluble additives from the [8]pristine plastic. In another experiment, the formation of biofilms on MP surfaces was induced by four weeks of incubation in seawater to make the material more attractive as a food source for the lugworm *Arenicola marina* (Gebhardt and Forster, 2018), an approach that can be likewise applied with soil solution. In order to remove soluble substances and fine particles from artificial MP, [8]pristine plastics have been treated with organic solvents (Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017b; Rodrigues-Seijo et al. 2018; Rodrigues-Seijo et al., 2019; Wang et al., 2019; Yang et al., 2019). If the plastic type is prone to the solvents, the surface is roughened by the dissolution of oligomers and, thus, increased. However, these techniques are not assumed to increase the number of carbonyl groups and surface charge. Therefore, they do not change the interaction with the soil matrix and the soil fauna, and have never been tested for similarity to natural weathering.

In contrast, some authors avoided artificial aging and instead applied natural weathering over shorter periods between two weeks and 12 month, which can be used as a kind of “plastic breeding” (e.g. Martin-Closas et al., 2016; Zhang et al., 2018). This treatment changes the physiochemical characteristics of plastics similar to environmental short-term weathering belowground and is suitable for the alteration of large amounts of plastic. But, it is very costly in terms of time when the production of strongly weathered MP is needed.

Once we know the important biogeochemical aging factors, long-term weathering experiments will be extremely helpful to understand the dynamics of surface alteration of soil MP. These experiments must take into account not only ecosystem parameters (e.g. humidity, edaphon activity and soil organic carbon) and start conditions such as plastic type, particle surface and protection by specific additives. The increase of surface area and charge density over time might cause a non-linear aging, while biofilm-cover cloaks the real MP surface characteristics – issues that should also be carefully included into the experimental design.

There is a great incentive to develop pre-weathering approaches to create designer-MP for laboratory experiments. Those close-to-nature weathering protocols might contain full chains of aboveground and in-soil aging factors and can be diversified according to actual material and environmental conditions. Applied to coming experiments, they will help us to better understand and predict short- and long-term effects of soil MP, the concentration of which is the result of decades of contamination and is still increasing.

Table 2: Approaches of surface (pre)weathering in recent experiments with soil microplastic. The abbreviations used in this table are as follows: UV – ultraviolet, TBBPA – tetrabromibisphenoal A, FE – feeding experiment. Polymers: BD – biodegradable plastics, OP – oxodegradable plastics, PA – polyamide, PE – polyethylene, PO – polyolefins, PP – polypropylene, PVC – polyvenyl chloride, TCE – thermoplastic copolyester elastomers. NA denotes that information was not available.

aging factor	applied plastic type	aging time (d)	resulting characteristics	experimental focus	reference
UV radiation (climate chamber)	diverse	variable	photooxidative aging	diverse	DIN EN ISO 4892-2, DIN EN ISO 4892-3
gamma irradiation (^{60}Co source)	PE, PP	NA	photooxidative aging	cation adsoprtion	Johansen et al. (2019)
discharged plasma oxidation (DPO)	PVC	0.02	photooxidative aging	TBBPA adsorption of and toxicity to algae	Zhou et al. (2020)
wather bath (90°C) + abrasion	PO, PA, PE, TCE	21	extraction of additives, increased accessibility for feeding organisms	feeding experiment with termites	Tsunoda et al. (2010)
incubation in seawater	PA, PS	28	surface biofilm formation	FE lugworms	Gebhardt and Forster (2018)
incubation in aquatic systems	PE, PP	19	surface biofilm formation	cation adsoprtion	Johansen et al. (2019)
methanol treatment	PE, PS	NA	extract soluble additives	FE earthworms	Wang et al. (2019)
ethanol treatment	PE	NA	extract soluble additives	FE earthworms	Rodrigues-Seijo et al. (2018)
	PE	NA	extract soluble additives	FE earthworms	Rodrigues-Seijo et al. (2019)
pentane + octane treatment	PE	NA	extract soluble additives	FE earthworms	Huerta Lwanga et al. (2016)
		NA	extract soluble additives	FE earthworms	Huerta Lwanga et al. (2017b)
		NA	extract soluble additives	FE earthworms	Yang et al. (2019)
plastic nursing (soil)	BD, OD, PE	~150	belowground weathering	mulch foil degradation experiment	Martin-Closas et al. (2016)
plastic nursing (soil, compost)	BD, PE	14-365	belowground weathering	feeding experiment with earthworms	Zhang et al. (2018)

Data availability

All of the data are published within this paper.

Author contributions

FB developed the article concept, collected data and prepared the manuscript. MK supervised the study by participating in structural discussions on the idea and concept of the paper and the final corrections.

Competing interests

The authors declare that they have no conflict of interest.

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