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Communication

Biodegradability of Disposable Surgical Face Masks Littered into Soil Systems during the COVID 19 Pandemic—A First Approach Using Microcosms

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Abstract: The COVID-19 pandemic caused massive use and improper disposal of surgical polypropylene (PP)-based face masks. For a first evaluation of the respective environmental consequences, we performed a 6-month microcosm experiment at 25 °C to determine the microbial degradability of 10 × 10 mm cuts of single mask layers and of a complete mask mixed with topsoil (Cambisol). By analyzing the CO_2 production, we identified a fast pool with a mean residence time (MRT_{fast}) of 3 to 7 days, corresponding to approximately 4 to 5% of the total mask carbon. Solid-state nuclear magnetic resonance (NMR) spectroscopy of the degraded masks suggests a cut-off of PP units or oligomers as a main degradation mechanism. The slow carbon pool of the center mask revealed an MRT_{slow} of 7 years and those of the remaining mask material MRT_{slow}s between 19 and 28 years, which is three to five times longer than those of soil organic matter (SOM) of the pure soil. Since the masks were not pretreated, and decomposed in the dark without UV radiation, our data support our hypothesis that in soils, microbes must exist that can decompose PP, although their nature still has to be revealed in future attempts.

Keywords: polypropylene degradation; Respicond apparatus; solid-state NMR spectroscopy; microplastic in soil

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1. Introduction

With the goal of limiting the disastrous impact of the COVID-19 pandemic, authorities of many countries recommended or even obligated the population to use face masks to stop the spread of this virus. Aside from being a precautionary measure to slow down the transmission rate from person to person [1], the use of face masks has helped in reducing the spreading of infection by avoiding that a person touches their face, mouth or nose with unwashed hands [2]. With the shortage of disposable masks at the beginning of the pandemic, the use of fabric-derived and often home-made masks started, but with the proceeding of the pandemic the worldwide production capacity of single-use face masks increased again to satisfy the request. In 2020, approximately 129 billion face masks per month were expected to be used worldwide [3] and 3.4 billion of those were estimated to be discarded every day [4].

The extended use of disposable face masks by the general population during the COVID-19 pandemic certainly contributed to decreasing infection rates, but at the same time created a further worldwide threat. The latter is associated with the increasing non-professional and inadequate disposal of used and unrecycled face masks along city lanes and sewage systems, but also on soils around public and private waste containers of dumpsites and landfills, as well as in rural and recreational areas [2]. Using the numbers provided by Prata et al. and Benson et al. [3,4] allows a rough estimation of the amount of

plastic litter which may have accumulated due to this behavior in soil environments during peak times of the pandemic. Accordingly, if during this time just 1% of the discarded single-use face masks with an average weight of 4 g (measured in our laboratory) had been littered improperly to the soil, an additional 136 tons of plastic waste would have entered the soil environment per day. Such thrown away masks endanger public health as they represent potential transportation media for invasive pathogens. As such, they jeopardize the health of waste collectors or litter pickers, but also members of the public. In particular, playing children who first come across the litter may be affected. Animals which unintentionally feed on their residues may become malnourished, as their stomachs are filled up with plastic residues without providing nutrition.

Most of the common disposable surgical face masks are manufactured of three layers of nanofiber plastic materials, mostly of polypropylene (PP) [5]. The inner layer consists of a fibrous material, the center layer is the melt gusted filter part and the outer layer, which is non-woven, is water resistant and colored. The material for the center (filter) layer is electrically charged to increase electrostatic adsorption of particles that otherwise could pass the filter. Littered into the environment, the masks are exposed to UV radiation, heat, moisture and physical abrasion, but may also suffer biodegradation effects, which add to their break down into smaller particles, microplastics and eventually into even smaller nanoplastics and fibers [6].

This deterioration or degradation of such masks and their break-down products is expected—and from a protective viewpoint also intended—to be slow. However, studies on the fate and longevity of surgical face masks in soils are scarce, although their micro- or nanoparticles may influence soil physical properties as well as microbial communities. Being transported through the soil both horizontally and vertically by bioturbation, cracking or plowing, impacts are expected at the soil surface but also in deeper soil regions. Widespread on and into the soil, they are likely to enter the food net with consequences for animal and human health that are still far from being well understood.

Investigating the biochemical recalcitrance of PP in a minimal media inoculated with a soil suspension extracted from a local plastic dumping site, it showed that only 0.4% of the plastic was decomposed after one year [7]. Cacciari et al. demonstrated the microbial biodegradability of PP films in a B7 medium by the formation of methylene chloride extraction products after 175 days of incubation [8]. They assumed that sulfate-reducing strains were involved in the oxidation of the PP. Based on the observation that lactate and glucose had to be added to the medium to promote PP degradation, they suggested that starch addition into various PP products could be a strategy to drive cometabolic processes. Longo et al. found decreasing crystallinity of PP films after their burial at 2 m in a sanitary landfill environment for 11 months [9]. This was explained with chain scission of the polymer chain. Arkatkar et al., on the other hand, observed the contrary in their experiments and attributed that to the preferential degradation of amorphous regions [7]. In a further study, Arkatkar et al. demonstrated growth of two Pseudomonas species and two Bacillus species, in particular *Bacillus flexus*, in the presence of untreated PP films [10]. Jeon and Kim isolated a mesophilic strain, *Stenotrophomonas panacihumi* PA3-2, from the soil of an open storage yard for municipal solid waste, and found that it degrades the low molecular weight fractions better than long-chain PP [11]. However, studies on the microbial degradation of PP are rarely performed after its addition to natural soils. In one of the few studies analyzing the fate of PP blended with starch, it was observed that biodegradation occurred mainly in the starch phase and not in that of PP [12]. The starch depletion created a porous matrix leading to a loss of structural integrity. Aside from adding blends with degradable additives [13], pretreatments with UV radiation or thermo-oxidation [14] seem to facilitate microbial degradation of PP.

Based the mentioned reports, we hypothesized that in soils, PP-based disposable surgical face masks can be biodegraded by microorganisms, although it may be at a low rate. This implies that with their increased disposal connected to their pandemic-caused ubiquitous and massive use, soils will suffer a considerable accumulation of partially

degraded mask residues which may cause a comparable environmental impact as does (micro)plastic from other sources. However, in spite of the economic importance and the widespread use of PP, neither possible degradation products nor mechanisms involved in its microbial degradation are sufficiently well understood [15] for an unbiased estimation of the longevity of PP-based disposable surgical face masks in soil. The latter, on the other hand, is essential for an unbiased evaluation of their potentially hazardous impact for such environment.

Therefore, to obtain a better idea of possible consequences of the inadequate disposal of those masks for soil systems, we started and report on a first attempt to assess their degradability by studying the decomposition of cuts of a complete mask and their three layers mixed into topsoil of a Cambisol under controlled conditions of a microcosm experiment. Complementary solid-state cross polarization magic angle spinning (CPMAS) ^{13}C nuclear magnetic resonance (NMR) spectroscopic analyses were conducted with the hope to identify chemical alterations that allow a better understanding of the involved mechanism. The analyzed masks were not pretreated or exposed to daylight before their incubation. By doing this, we excluded any effects by previous physical or chemical alteration, for example by UV radiation, heat or moisture, and ensured that only biodegradability of the masks was assessed.

2. Materials and Methods

2.1. Microcosm Experiment

Monitoring the physical degradation of an unintentionally disposed surgical face mask on a fallow close to Seville, Southern Spain, for approximately one year confirmed progressing physical destruction of the mask (Figure 1). An ant colony settled close by, and a transport of little mask pieces by ants into the nest was observed.

Figure 1. Surgical face mask, unintentionally deposited on a fallow close to Seville in Southern Spain during its natural degradation. The photos were taken on (**a**) 16 December 2020; (**b**) 11 August 2021; (**c**) 22 October 2021 and (**d**) 4 January 2022. In order to avoid dislocation of the mask, i.e., by wind, the strings were fixed with stones at the start of the monitoring (**a**). However, the stones were removed by unknowns between August and October 2021.

Based on such observations, we felt that it was justifiable to use mask material which was cut into small pieces for our experiment. As shown in Figure 2, in a first step, the strings of commercially available single-use face masks (Zhangjiagang mingyu new material technology co., Ltd., Zhangjiagang, China) were removed and their layers were separated into outer (O), inner (I) and center (C) mask.

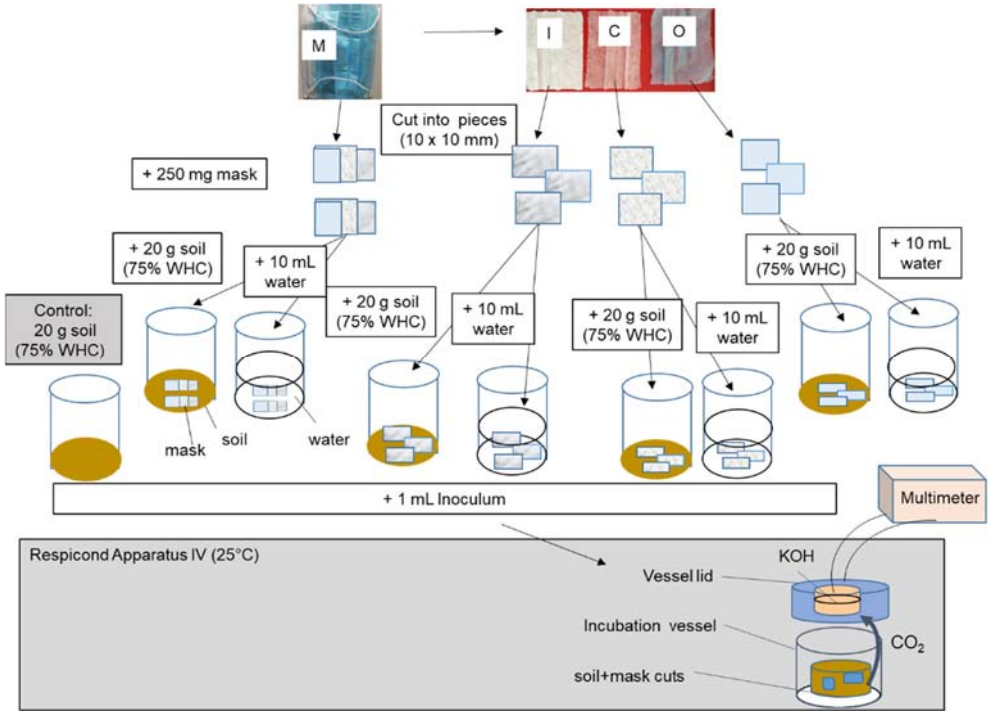

Figure 2. Scheme of the experimental design of the microcosm experiment for the study of the microbial degradation of surgical face mask residues in a soil environment. As a control, only soil was incubated after inoculation at 75% WHC.

Subsequently, the layers were cut into pieces of approximately 10 × 10 mm. In addition, complete masks (M) but without the strings and without separating the different layers were cut correspondently. For each mask type, eight glass beakers of 100 mL were filled with 20 g soil derived from the A horizon of a Cambisol from the Sierra de Aznalcóllar, Seville, Southern Spain. A more detailed description of soils of this area is given in Lopéz-Martín et al. [16]. As a control, beakers were filled with only 20 g of soil without mask material. The used soil with a pH of 6.3 has an organic carbon (C) and nitrogen (N) content of 43.7 mg g^{-1} and 3.6 mg g^{-1}, respectively. Thus, 950 mg of organic C was added to the sample with the soil (soil organic carbon: SOC). The carbon concentration of the mask was determined via elemental analysis and was the same for each layer. Therefore, addition of 250 mg of mask pieces with a C content of 860 mg g^{-1} added 215 mg of organic C (mask organic carbon: MOC) to the soil, which corresponds to 18% of the total C of the mixture. Thereafter, the control soils and the blended soils were inoculated with 1 mL of a filtered (Whatman paper filter) suspension containing a diverse but undefined microbial consortium extracted from 10 g of gardening soil in use, mixed with 100 mL distilled water. This approach was used to ensure that most of the common soil microorganisms are present. A more detailed analysis of the single species was not conducted, since the present study focused on the chemical aspects of the degradation of the face masks rather than the identification of the participating organisms or enzymes. Subsequently, the soil–mask mixture was moistened to a degree which corresponds to 75% of the maximum water holding capacity (WHC) of the soil, determined prior to the experiment according to Veihmeyer and Hendrickson [17]. The mask cuts and the soil were carefully mixed to ensure that all mask cuts were covered with moist soil. The soil depth in the beaker was approximately 1–1.5 cm, which ensured

good soil aeration and prevented leaching of degradation products into subsoil regions. In addition, 8 replicates of 250 mg masks cuts were incubated with 10 mL distilled water and 1 mL inoculated water but without soil.

All beakers were randomly placed into closed plastic vessels of a Respicond Apparatus IV (Nordgren Innovations, Umea, Sweden) and incubated in the dark at 25 °C (room temperature). The C loss due to microbial degradation in each vessel was determined every 6 h by measuring changes of the electrical conductivity of a 0.6 M KOH solution which were caused by the absorption of emitted CO_2 [18]. This solution was located in a small, perforated plastic vessel fixed on the top of the vessel lid and equipped with platinum electrodes.

After 1 month, four replicates were removed from the Respicond Apparatus IV of which two were dried at 40 °C for their NMR spectroscopic characterization and the remaining two were stored at −80 °C for future analysis. After 6 months the remaining 4 replicates were also removed and treated comparably.

For a first evaluation of the degradability of the masks, the means of C released from each of the remaining four replicates of the control soil and of the mask–soil mixtures were calculated for each measurement point and plotted as functions of incubation time for a period of six months. The data collection for the water–mask mixture experiment was aborted after 1 month due to insufficient CO_2 production. The mean C-release values were subsequently fitted to a double exponential decay model (pools with fast and slow turnover) using Sigmaplot 14.0 (Systyt software, Inc., San Jose, CA, USA) according to Equation (1) [19]. Fits applying a triple exponential decay model did not pass the constant variance test.

$$C(t) = A_{fast} \times e^{-kfast\ t} + A_{slow} \times e^{-kslow\ t} \quad (1)$$

In Equation (1), t = incubation time; $C(t)$ = remaining C (% of total C) at t; A_{fast} = amount of C which is relatively labile against mineralization (% of total C); A_{slow} = amount of C which is more stable against degradation (% of total C_{org}); k_{fast} and k_{slow} = apparent first-order mineralization rate constants for the labile and refractory pool (y^{-1}). Accordingly, the mean residence times (MRT_{fast} and MRT_{slow}) of the first-order reactions are equal to $1/k_{fast}$ and $1/k_{slow}$.

Characterization of the soil organic matter (SOM) in the soil incubated with and without mask material by solid-state NMR spectroscopy indicated that addition of the masks to the soil did not alter the humification of SOM. Therefore, the degradation parameters of the mask material were obtained by fitting the curves from values calculated from the difference between the C-release of the soils with and without mask addition for each measured point.

2.2. Solid-State CPMAS ^{13}C NMR Spectroscopy

For the solid-state ^{13}C NMR spectroscopic detection of chemical alterations of the fresh mask during microbial degradation, randomly taken cuts from each layer of a facial mask were torn with tweezers into little fibers and filled into a CRAMPS zirconium rotor of a diameter of 4 mm containing an inlet made of KEL-F®. The mask cuts in the incubated soils were handpicked and roughly cleaned from the soil. No water or solvent was used, which avoided leaching of residual mask fibers. A complete removal of the residual soil was not necessary, since due to its low carbon content its contribution to the ^{13}C intensity was below the detection range. Each layer was probed three times by renewing the sample material for each measurement. Using a Bruker 400 MHz, Advance III HD spectrometer with a triple resonance wide bore probe, all spectra were obtained with the cross-polarization (CP) technique using a contact time of 1 ms and applying a magic angle spinning (MAS) rate of 14 kHz. Pre-experiments with variable contact time settings confirmed that this contact time is appropriate to obtain quantitative data [20]. The recycling delay was adjusted to 5 s, which corresponds to 5 times of the longest ^{1}H-spin-lattice relaxation rate constant, T_{1H}, determined for the different peaks in the spectra of each mask layer applying the inversion recovery pulse sequence. Due to measurement time restriction, T_{1H} could not be performed

for each triplicate of a sample set. As a consequence, ANOVA was not applied. However, in order to detect a tendency, duplicates of selected sample sets were analyzed, and their standard deviation determined.

2.3. Statistical Design

Data normality was checked with a Shapiro–Wilk's and Kolmogorov–Smirnov tests. A one-way analysis of variance (ANOVA) was performed to compare the impact of mask type on C-release (n = 7 and n = 4) and their chemical alteration (n = 3) during their incubation. Post hoc multiple pairwise comparisons were carried out using a Tukey's HSD (honestly significant difference) test at a p = 0.05 significance level. All the statistical analyses were performed using IBM SPSS Statistics 26 software.

3. Results and Discussion

3.1. Composition of the Starting Mask Material

The signals at 21 ppm, 26 ppm and 44 ppm in the ^{13}C NMR spectra of all three mask layers before incubation are typically for fibers of isotactic polypropylene [21] (Figure 3). Other signals are not detectable, indicating that impurities play a negligible role. Applying a one-way ANOVA, no significant difference of the chemical composition of the three layers was evidenced (Table 1). Comparable T_{1H}-values between 1 and 1.1 s were determined for all signals of all layers (Table 2). Since T_{1H} is correlated with molecular motions in the MHz timescale regime [22], the observed values of the mask materials indicate high crystallinity of their polymer.

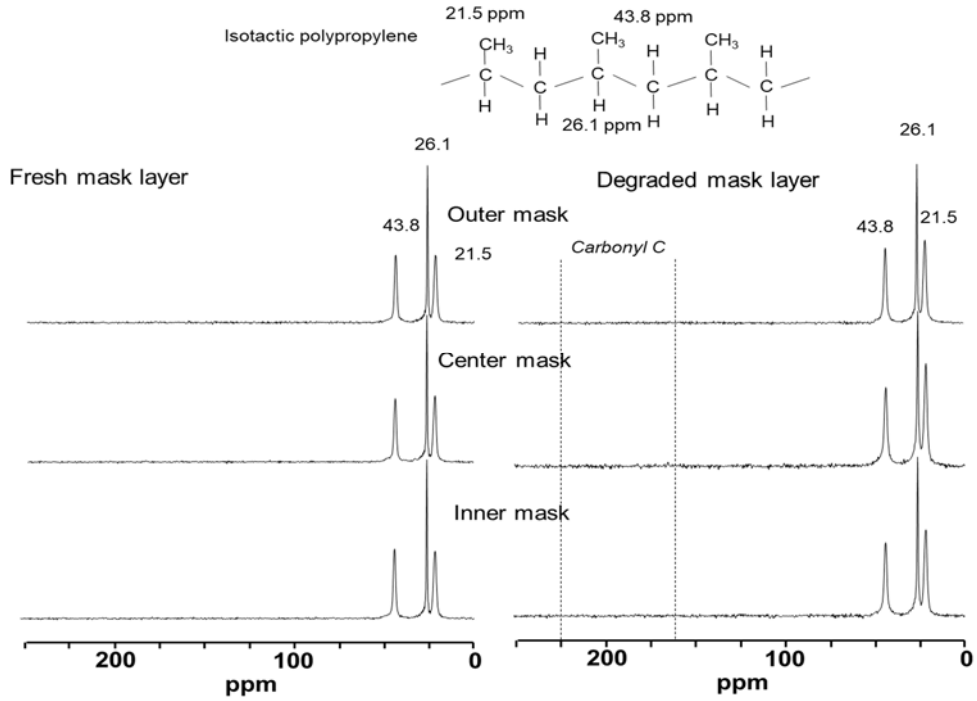

Figure 3. Representative solid-state CPMAS ^{13}C NMR spectra of the different layers of a 3-layered polypropylene-based surgical face mask before and after 6 months of microbial degradation in the topsoil of a Cambisol with a moisture of 75% of the maximal water holding capacity. The chemical shifts of the different resonance lines are indicated by numbers above the signal. Their intensities are listed in Table 1 as the mean of three NMR measurements of three sample replicates together with their standard deviations.

Table 1. Intensity distribution (% of total intensity) in the CPMAS ^{13}C NMR spectra of the outer, center and inner layer of single-use face masks before (t0) and after 1 and 6 months (t1, t6) of incubation as cuts from a single layer (S) or from a complete mask (M). The values are means ± standard deviations of three NMR measurements obtained from independent replicates. For each chemical shift region, the numbers followed by the same letter indicate no significant differences at the $p < 0.05$ level.

	50–40 ppm			30–24 ppm			24–15 ppm		
	Outer	**Center**	**Inner**	**Outer**	**Center**	**Inner**	**Outer**	**Center**	**Inner**
St0	32.6 ± 0.0 a	32.6 ± 0.2 a	32.6 ± 0.1 a	32.8 ± 0.1 a	32.8 ± 0.2 ab	32.8 ± 0.1 a	34.6 ± 0.0 a	34.7 ± 0.4 a	34.6 ± 0.1 a
St1	31.7 ± 0.1 ab	31.0 ± 0.4 bc	31.4 ± 0.4 bc	32.0 ± 0.3 abc	31.5 ± 0.3 c	31.7 ± 0.3 bc	36.6 ± 0.4 ab	37.5 ± 0.7 bc	36.9 ± 0.7 b
Mt1	31.4 ± 0.1 bc	31.8 ± 0.2 ab	31.7 ± 0.1 ab	31.8 ± 0.3 abc	32.1 ± 0.2 abc	31.8 ± 0.8 abc	36.8 ± 0.3 b	36.1 ± 0.3 ab	36.5 ± 0.8 ab
St6	30.9 ± 0.6 bc	28.9 ± 0.6 d	30.5 ± 0.3 c	32.9 ± 0.5 a	32.0 ± 0.3 abc	32.5 ± 0.5 abc	36.2 ± 1.1 ab	39.1 ± 0.4 c	37.0 ± 0.9 b
Mt6	30.5 ± 0.6 c	31.4 ± 0.7 bc	30.8 ± 0.6 c	32.8 ± 0.5 a	32.9 ± 0.1 a	32.7 ± 0.2 ab	36.7 ± 1.1 b	35.6 ± 0.8 ab	36.5 ± 0.7 ab

Table 2. ^{1}H-Spin-lattice relaxation time T_{1H} (ms) of carbons in the polypropylene polymer of the outer, center and inner layer of single-use face masks before (t0), after 1 month (t1) and 6 months (t6) of incubation as cuts from a single layer (S) or from a complete mask (M). Standard deviations between 1 and 37 ms were determined for duplicates.

	50–40 ppm			30–24 ppm			24–15 ppm		
	Outer	**Center**	**Inner**	**Outer**	**Center**	**Inner**	**Outer**	**Center**	**Inner**
St0	1057	1086	994	1038	1065	1031	1021	1066	1060
St1	1029	1048	1021 * ± 35	995	1019	1027 * ± 23	985	1007	1010 * ± 19
Mt1	1057	996	1010	1017	987	996	945	984	1008
St6	910 * ± 12	961 * ± 31	955 * ± 26	969 * ± 1	970 * ± 16	933 * ± 37	948 * ± 4	984 * ± 29	931 * ± 24
Mt6	1027 * ± 8	960	959	994 * ± 36	943	950	1017 ± 32	920	949

* Average value obtained from two replicates and per standard deviation.

3.2. Microbial Decomposition of Mask Material

After one month, the control soil lost 46.6 ± 1.8 mg C as CO_2, which corresponds to 5% of its initial total C (Ct_{soil}) (Table 3). Here, it should be mentioned that the amount of C released from the soil is commonly smaller than the total amount of C affected by microbial degradation, since some of this C is recycled for the build-up of new biomass [19]. After 6 months, 13% of the Ct_{soil} was released as CO_2.

Table 3. Carbon (C) loss of total C of soil–mask mixtures (Ct_{sample}) (mg and % of Ct_{sample}) after 7, 31 and 178 days of incubation in a RESPICOND Apparatus IV at 25 °C. The C loss of the respective masks (mg) was calculated from the difference of the loss in the pure soil sample and the soil–mask mixtures (mg) and related to the total C of the mask (Ct_{mask}) before incubation. Ct_{soil} describes the carbon content of the pure soil.

	C_{sample} Loss (mg)			C_{sample} Loss (% of Ct_{sample})			C_{mask} Loss (mg)			C_{mask} Loss (% of Ct_{mask})		
Incubation Time (Days)	**7** [1]	**31** [1]	**178** [2]	**7**	**31**	**178**	**7**	**31**	**178**	**7**	**31**	**178**
Soil (S)	17.0 ± 1.3 a	46.6 ± 1.8 a	119.4 ± 1.6 a	1.8	4.9	12.6						
S/outer mask	25.4 ± 1.9 b	58.1 ± 3.0 b	132.9 ± 9.1 b	2.2	5.0	11.4	8.4	11.4	13.6	3.9	5.3	6.3
S/center mask	25.4 ± 1.5 bc	57.9 ± 2.6 b	141.8 ± 5.1 b	2.2	5.0	12.2	8.4	11.3	22.4	3.9	5.2	10.4
S/inner mask	26.4 ± 1.4 b	57.5 ± 2.5 b	133.4 ± 6.2 b	2.3	5.0	11.5	9.5	10.9	14.0	4.4	5.1	6.5
S/complete mask	22.5 ± 3.6 c	52.7 ± 6.3 b	132.5 ± 3.0 b	1.9	4.5	11.4	5.5	6.1	13.1	2.6	2.8	6.1

[1] Values in the columns are means ± standard deviation with $n = 7$ and [2] $n = 4$. Same letters (a–c) following the numbers of a column indicate no significant differences at the $p < 0.05$ level.

The addition of mask cuts of the single layers to the soil significantly increased CO_2-C release after 1 month by 24% and added up to 58 mg. Note that this increase is not explainable by priming alone. After 6 months, the difference of the amount of produced CO_2 between the pure soil and the soil–mask mixtures was still evident. For the soils with the outer and inner mask, the additional C-release corresponded to 12% of that observed for the pure soil. For the center mask, this value even reached 20%. However, visual inspection

of the mask cuts after one-month degradation time did not show major alterations, but most of the cuts stuck together with soil material, suggesting that at least some interactions between mask and soil occurred. On the other hand, after 6 months, clear signs of physical deterioration were observed, in particular for the center masks. This was also indicated by the fact that tearing the fibers apart with tweezers was easier than it had been for the original mask material.

Based on the extent of C that was additionally released in the samples with mask addition and the fact that an NMR spectroscopic analysis of the pure soils incubated with and without mask did not show notable differences (data not shown), we concluded that priming of SOC degradation due to mask addition has negligible impacts in our experiment. Therefore, the additional C loss for the soil mixed with the mask material has to be attributed to the decomposition of the masks. Accordingly, approximately 5% and 3% of the C (Ct_{mask}) of the single mask layers and the complete mask cuts were lost already during the first month of degradation, which increased to 6 to 10% after 6 months (Table 3). Most of this loss (18 to 31%) occurred during the first incubation week, which clearly demonstrates the presence of a small C-pool with high microbial accessibility. However, most of the mask C belonged to more stable C-pools with slow mineralization rates.

The C-release from mask cuts covered only with water was in the range of 1.8 mg, corresponding to 0.8% of Ct_{mask} after 1 month of incubation. This is in line with the previously observed high persistence of single-use masks in aquatic systems [23]. However, lack of nutrients may have contributed to the low decomposition rate.

For a more detailed analysis of the mineralization rates of masks in soil environments, the C-release for four replicates of each mask–water and mask–soil mixture was determined for a period of three and six months, respectively. Figure 4 gives an example of the curves obtained for the averaged values from the control soils (A), the soils mixed with cuts of complete masks (B) and the difference between A and B. Whereas for the soil and the mask–soil mixtures curves were obtained that could be fitted with a two exponential decay model, the C-release of the mask–water mixture was too low for receiving unbiased mineralization rate constants.

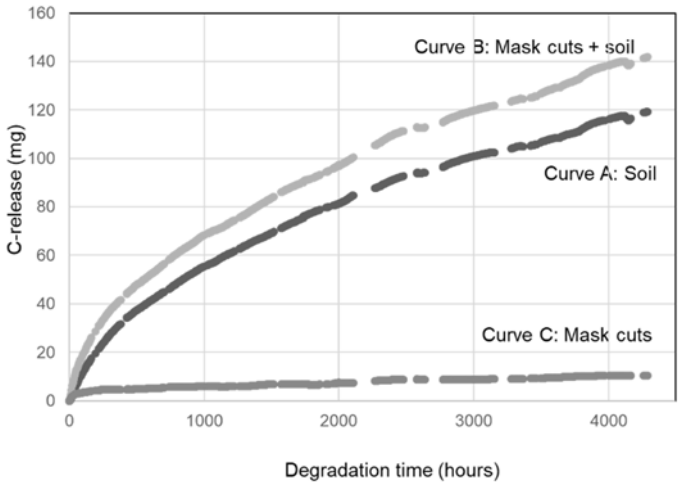

Figure 4. C released as CO_2 from a topsoil of a Cambisol incubated at a moisture of 75% of the maximal water holding capacity without (curve A) and with (curve B) addition of cuts from complete masks as a function of degradation time. Each point is the mean of four replicates. The C released only from the mask cuts (curve C) was obtained from the difference between curve A and B.

As shown in Table 4, the fits for the soil and the masks revealed a small fraction between 4 and 5% of their total C (Ct) that was quickly mineralized to CO_2 (A_{fast}). Note that the MRT_{fast} of the soil C was, with 42 days, approximately 6 and 14 times longer than those of the C of the mask cuts of the complete mask and of the single layers, respectively. The mineralization rate constant k_{fast} for A_{fast} of the soil lies in the range of slowly degradable

biopolymers such as hemicellulose [19]. However, most of the SOM (94%) decomposed at a rate typically found for partially humified material [19]. Its k_{slow} of 4×10^{-4} days^{-1} corresponded to an MRT_{slow} of 6 years, which is shorter than the MRT_{slow} previously determined for other soils of the same region [24]. Comparing this time with those of SOM of other soils, one has to bear in mind that our approach conducts a simple fractionation of SOM into a fast and a slow turning pool without considering the high heterogeneity of SOM. In particular, the slow fraction has to be seen as a mixture of compounds that survived the first fast mineralization but still contains constituents with different mineralization rate constants. Since MRT_{slow} reflects an averaged value, seasonal variation of the amount and degradation state of litter residues are altering the observed MRTs and may be responsible for the difference between the values obtained here and in the former study. Extrapolating the obtained values to natural conditions, one has also to bear in mind that our mineralization rates were determined under the optimized conditions of a laboratory microcosm experiment. Natural conditions such as seasonal changes of soil temperature or soil moisture are likely to increase MRT, at least in temperate or dry climatic zones.

Table 4. Contribution of the fast (A_{fast}) and slow (A_{slow}) turning pools of the A horizon of a Cambisol and different layers of single-use face masks amended to this Cambisol, their degradation rate constants (k_{fast}, k_{slow}) and the respective mean residence times (MRT_{fast}, MRT_{slow}). The data derive from a two-component exponential decay model fitted to the mean CO_2 production of four replicates incubated for 6 months.

	A_{fast} %	k_{fast} Days^{-1}	MRT_{fast} Years	MRT_{fast} Days	A_{slow} %	k_{slow} Days^{-1}	MRT_{slow} Years	R^2
soil	5.2	0.02	0.114	41.7	94.4	0.00043	6	0.999
outer mask	4.4	0.40	0.007	2.5	95.1	0.00014	19	0.693
center mask	3.8	0.34	0.008	3.0	95.7	0.00040	7	0.981
inner mask	4.7	0.38	0.008	2.8	94.8	0.00010	28	0.952
complete mask	4.0	0.14	0.019	6.9	95.1	0.00013	21	0.946

The k_{fast}s of the mask cuts determined from our experiment are comparable to biochemically labile organic matter such as hemicellulose [19]. They may derive from short amorphous PP-chain residues that are easily accessible to microbes. After their decomposition, the remaining 95 to 96% of the Ct_{mask}s were mineralized with considerably slower rate constants (1 to 4×10^{-4} days^{-1} corresponding to 0.04 to 0.14 year^{-1}) than the slow fraction of the soil. However, they are still in the range found for humified SOM in A horizons of other Cambisols in the sampling region [24]. Note that the center (filter) mask is less resistant against mineralization than the outer and inner mask. Calculating their MRT_{slow} suggests that under the controlled conditions of the laboratory, the C of the first remains in the soil for at least 7 years and that of the other layers for at least 19 to 28 years. Although the amount of C_{mask} which was released within 6 months (Table 3) is with 6% to 10% of Ct_{mask}, lower than the 13 to 20% observed by [11] for powdered PP films decomposing in compost at 37 °C for 90 days, our results give further evidence that microorganisms naturally occurring in soils can degrade PP. Other studies report that Lysinibacillus sp. reduced the weight of PP by 4% over a time frame of 26 days [25]. Bearing in mind that PP is not UV resistant, the exposition of the masks to daylight before their incorporation into the soil is likely to speed up subsequent biotic decomposition within the soil [26,27]. On the other hand, in addition to seasonal climatic alterations, unfavorable conditions for microbial activity or transportation to deeper soil regions with reduced oxygen availability affect microbial activity, which may increase the longevity of masks in such environments.

3.3. Chemical Changes during Microbial Degradation of Single-Use Face Masks

The chemical changes during the decomposition of the cuts of the single layers incubated for 6 months (St6) were investigated by comparing the intensity distribution of their

solid-state ^{13}C NMR spectra with those of the untreated mask (Table 1). In order to reveal if the separation of the different layers affect the degradation pattern, the layers of the incubated cuts of the complete mask (Mt1, Mt6) were separated and analyzed individually. For all C groups in all samples except the outer layer of the cuts of the complete mask, a tendency of shortening of T_{1H} with degradation time (Table 2) was detected, indicating that microbial degradation increased intramolecular mobility within the polymer. This can be related to decreased crystallinity caused by degradation or deterioration, as it was also observed in other studies [6]. However, in our case, neither UV radiation nor physical abrasion can be made responsible for the decreasing crystallinity, suggesting that biochemical or chemical reactions are the major players. The fact that incubation did not change the chemical shifts of the signals (Figure 3) suggests further that the monomer structure of the polymer remained widely unaltered. Different from studies of abiotic degradation by UV oxidation [27] and thermal pretreatment [7], no indication for the formation of carbonyl C was evidenced. Possibly, accumulation of carbonyl-C-containing metabolites was prevented by their high accessibility to enzymatic processes, thus to quick metabolization.

Analyzing the relative intensity distribution of the spectra by one-way ANOVA confirmed slight but statistically significant changes of the chemical composition due to microbial degradation for the single masks (St6) after 6 months (Table 1). They are most pronounced for the center mask cuts. Changes were also observed for the cuts of the complete mask, although they are less expressed. The alterations resulted in a relative increase of methyl C (21.5 ppm) at the expense of the tertiary and secondary C.

For further analysis of the chemical changes, it was assumed that on a molecular level, the chemical composition of the analyzed mask cuts was representative for all mask remains (including possible leachates into the soil environment) in the soil–mask mixture, and that only negligible amounts of mask C were incorporated into microbial biomass. With this simplification, the absolute amount of remaining C causing intensity in the different chemical shift regions of the spectra of the cuts of the single mask layers after 1- and 6-month degradation (Table 5) was calculated by using the respective C losses as they were determined from the mineralization curve (Table 3). Thus, the total intensity in the spectra of the degraded masks after 1 and 6 months of incubation listed in Table 1 corresponds to 95–97% and 90–94% of Ct_{mask}.

Table 5. Remaining Ct_{mask} (%) in C-types of PP of the outer, center and inner layer of single-use face masks incubated with topsoil either as cuts of single layers (S) or of complete masks (M) for 1 and 6 months (t1; t6) under the controlled conditions of a microcosm experiment. The numbers were calculated from the mean values of the relative intensity in the chemical shift regions of the CPMAS ^{13}C NMR spectra of the undegraded mask layers (St0) and the C loss determined by CO_2 production during incubation (Table 3).

	50–40 ppm			30–24 ppm			24–15 ppm		
	Outer	**Center**	**Inner**	**Outer**	**Center**	**Inner**	**Outer**	**Center**	**Inner**
St0	32.6	32.6	32.6	32.8	32.8	32.8	34.6	34.7	34.6
St1	30.1	29.5	29.6	30.3	29.9	30.0	34.7	35.6	35.4
Mt1	30.5	30.8	30.7	30.9	31.2	30.9	35.7	35.0	35.4
St6	29.0	25.7	28.7	30.9	28.5	30.5	34.1	34.8	34.8
Mt6	28.7	29.6	28.9	30.8	30.9	30.8	34.5	33.5	34.3

This estimation confirmed a larger net loss of secondary and tertiary C than of CH_3 (Figure 5). Together with the decreasing crystallinity, the detected pattern points towards a cutting of the polymer into smaller units and a subsequent split-off of terminal propylene units which are further mineralized to CO_2.

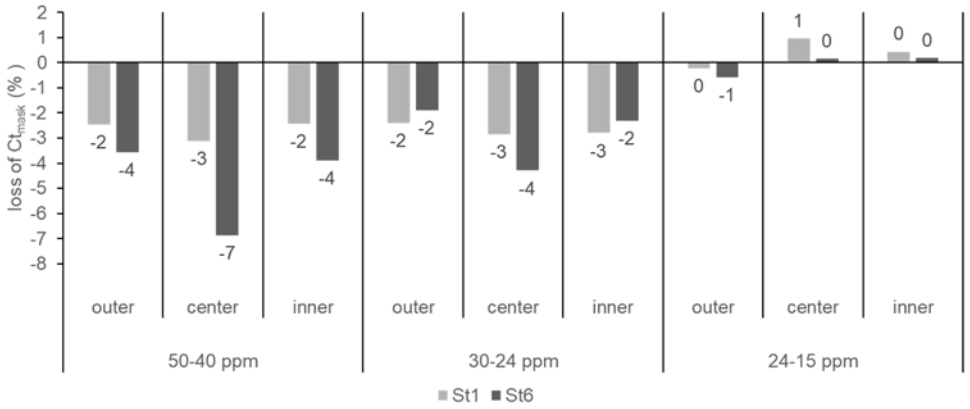

Figure 5. Net C loss of Ct_{mask} for each C-type (%) calculated from the difference between Ct_{mask} in the fresh and degraded masks (Table 5) that were incubated as cuts from single layers for 1 and 6 months (St1, St6). Negative values are net losses; positive values correspond to net production.

4. Conclusions

Although our preliminary study describes only the first six months of surgical single-use face mask decomposition, we were able to confirm our hypothesis that microorganisms present in natural soils without any waste disposal history can decompose PP and are involved in its biotic decomposition. However, in line with the obvious accumulation of single-use masks in oceans, rivers or lakes, those microorganisms seem to work inefficiently if the medium is only water. If this is caused by the high hydrophobicity of the masks, oxygen depletion or the lack of adequate nutrients remains an open question. The studied face masks contained a very quickly mineralizable fraction accounting for 4 to 5% of their total carbon, the degradation of which did not visually alter their appearance but still released CO_2. The calculated MRT_{slow}s between 7 and 28 years determined in our microcosm experiment are longer than those determined for the slow fraction of the natural SOM but still do not support extremely long residence times of mask residues in natural soils. However, their persistence is certainly sufficiently long for posing a threat to the environment, allowing the transport of only partially degraded residues to rivers and oceans or their incorporation into the soil system. Here, it may change soil properties and soil processes. Both in aquatic and in terrestrial systems, the survival of mask residues can be dangerous for wild animals that take them up as or with their food. Note that this study is only a first attempt in the need for a better understanding of the consequences of the massive mask littering during the COVID-19 pandemic for terrestrial ecosystems. Further studies are needed in which not only the impact of environmental conditions, plant growth or previous exposure to UV radiation on mask degradability is studied, but also in which the effect of the latter on the delicate ecological equilibrium in soils is assessed.

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