Development and application of tests for microplastic detection in soil - Fabio Alfonso Corradini Santander

Development and application of tests for microplastic detection in soil

Fabio Alfonso Corradini Santander

Propositions

1. The term 'microplastics' is an abstraction that hampers the development of analytical methods designed to understand their behaviour in the environment.
   (this thesis)
2. The use of sludge as fertilizer to recover nutrients in circular systems constitutes an environmental threat wherever ecosystems boundaries are poorly understood.
   (this thesis)
3. Postgraduate studies and the academic career articulate a pyramid scheme that makes PhD students' working conditions precarious and vulnerable.
4. Publishing houses and global indexing of research and researchers have reduced science to an ego contest, strongly focused on end products, that deprives researchers of thinking, writing, and reading at peace.
5. Free, open, and spread access to high quality education and information is a *sine qua non* to overcome poverty.
6. The greatest duty parents have is to educate their children and teach them about empathy and tolerance.

Propositions belonging to the thesis entitled

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Fabio Corradini Santander

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Development and application of tests for microplastic detection in soil

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Chapter 1. General introduction

1.1 Research on plastics in the environment — a glance at the story so far

Plastics serve as versatile and malleable matrices that manufacturers can easily manipulate in an effort to develop new technologies that will benefit society. These new technologies allow manufacturers to produce products that customers can use either a single time or, in some cases, for more than 50 years. Due to its great versatility, industries across all sectors use plastic at some point during the production process or include plastics in the final products. Global plastic production reached 359 million tonnes in 2018. In Europe alone, the demand for plastics in 2018 hit 51.2 million tonnes (PlasticEurope, 2019). The packaging industry accounted for 40%, construction for 20%, and the automotive industry for 10% of this demand. That comes out to nearly 20.5 million tonnes of plastic used in packaging in Europe alone. Since packaging belongs to the group of disposable plastic products, circular economists and environmental scientists wonder about the end of life phase of plastics. At a glance, the problem shouldn't really pose a significant threat. In 2018, 29.1 million tonnes of plastics were collected for recycling. However, upon closer inspection, one can see that the amount of plastic that is collected does not necessarily reflect the volume of plastic waste that is produced. The amount of plastic that is improperly disposed of isn't officially recorded (PlasticEurope, 2019). Global estimates indicate that consumers discard at least 2% of their plastic waste improperly by throwing it away directly in the environment where it becomes 'plastic litter' (Jambeck et al., 2015). This statistic includes all countries, as littered plastic waste includes all plastics that are dumped without consent in inappropriate locations (e.g. cigarette filters, plastic bottles, food packages). In Europe in 2018, the 2% stood for approximately 582 tonnes of plastics that were inappropriately disposed of. As we move from the high-income countries of Europe to lower income countries throughout the world, plastic disposal becomes a more complex problem(Wilson et al., 2015).

One of the most striking socio-economic factors, global inequality manifests itself through a country's waste management strategies. More affluent countries have low rates of inadequate or uncontrolled disposal of plastic waste, between 0% and 2%. In lower income countries, uncontrolled disposal of plastics is much higher with 36% in lower-middle income countries and 65% in low income countries (65%) (Wilson et al., 2015) (Figure 1.1). For these countries, inadequate disposal does not include plastic litter. The term 'inadequate disposal' describes plastic waste that is disposed of without formal management which includes disposal in dumps or open landfills that have containment flaws. The United Nations examined the relationship between a country's GDP and its waste collection coverage, pointing out that collection coverage reached only 36% of the population in low income countries (Wilson et al., 2015). To digest this number correctly and understand its implications, the reader should keep in mind another facet of inequality. The demand for

plastics in low and lower-middle income countries is only one fifth of the demand of their richer counterparts. In other words, developed countries produce more waste than developing ones, reducing the gap between the net contribution that countries make to the plastic pollution problem (Ritchie, 2018).

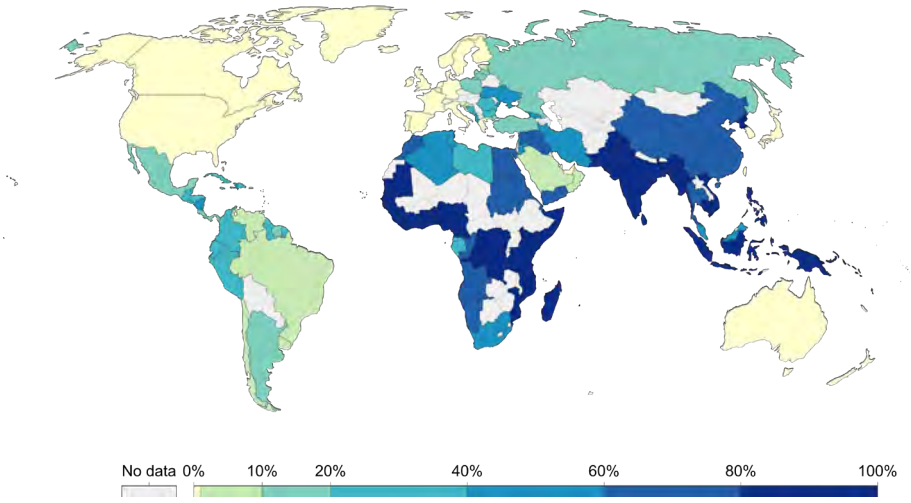

Figure 1.1. *World data: Rate of uncontrolled plastic disposal by country. Adapted from (Ritchie, 2018).*

However different the disposal scenarios can be, all countries contribute to the global problem of plastic waste management (Figure 1.2). In 2010, the volume of global plastic production was a mere 25% of today's total —according to PlasticEurope (2019) data. At the time, scientists estimated that 8 out of the 32 million tonnes of mismanaged plastic waste entered the oceans (Jambeck et al., 2015). In the same year, terrestrial ecosystems received approximately 24 million tonnes of mismanaged plastic waste. These statistics are unsettling considering the fact that global production of plastics increases every year, as does the volume of plastic waste. Where is all of this plastic waste ending up?

For many years, researchers thought that the ocean acted as the primary global plastic sink. The idea probably came about because the plastic that floats is easily seen and acknowledged while the plastic that is buried is quickly forgotten. In the early 1970's, scientists first theorized about the environmental consequences of plastics and the threat that biomagnification of associated plasticizers posed to human health (Carpenter and Smith Jr, 1972). In that first scientific report, the authors described 0.25 to 0.5cm buoyant particles found in the Sargasso Sea. Subsequently, oceanographers began to accumulate evidence concerning the plastic pollution in aquatic ecosystems. During this first period of

discovery, scientists saw microplastics as buoyant plastic pellets and ignored any specific particle size or composition. While both large and small plastic particles have been studied since the beginning, scientists quickly placed the main focus on the large plastic particles.

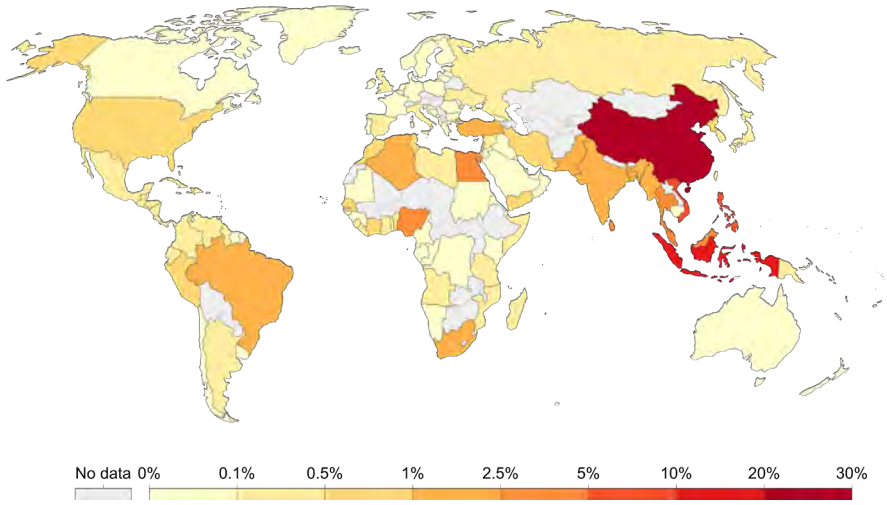

Figure 1.2. *World data: Countries' share of global mismanaged plastic waste. Adapted from (Ritchie, 2018).*

It took another 30 years for research on microplastics in aquatic environments to take off. In 2004, an article published in Science warned that microplastic pollution was a common, ubiquitous, and expanding phenomenon that threated ecosystem integrity (Thompson et al., 2004). The study by Thompson et al. (2004) was the first to use the term "microplastics" in regards to the marine environment. However, the authors did not propose a formal definition. The first definition emerged in 2008 at the *"International research workshop on the occurrence, effects, and fate of microplastic marine debris"* hosted by the University of Washington Tacoma (Arthur et al., 2008). At the workshop, attendees agreed that microplastics included plastic fragments measuring less than 5mm. The definition carried a twofold problem. First, it neglected the composition of microplastics. Second, it ignored the need for a classification system to correlate information about sources, shapes, and materials. As a result, even today, whenever scientists study microplastics they struggle with flawed analytical methods that do not fit the plethora of shapes and compositions 'microplastics' could have (Table 1.1). In other words, the initial definition poses several challenges for measuring and monitoring microplastic occurrence, fate, and effects in the environment (section 1.5).

Table 1.1. *A glance at what scientists mean by 'microplastics': a pollutant with different shapes, sizes and origins that puzzle analysts who try to trace its fate in the environment.*

Shapes	Polymer	Origin	Size ranges	Review
Tire wear particles	Poly butadiene Styrene butadiene Polysulphide Neoprene isoprene ...	Tires	On-road driving [0.5 – 20 um] Road runoff [1 - 100 um]	(Wagner et al., 2018)
Synthetic fibers	Polyamide Polyester Polyacrylonitrile Polyethylene Polypropylene ...	Textiles Fishing nets Ropes	Laundry (washing) [25 – > 3,000 um] Laundry (drying) [19 – 4,000 um] Industrial uses [<15 – 20 um]	(Carney Almroth et al., 2018) (O'Brien et al., 2020) (Du et al., 2020a)
Fragments	Polypropylene Polystyrene Polyethylene Polyvinyl chloride ...	Extrusion leftovers Disposable plastics Building materials Plastic tools	Extrusion [0.001 – 0.21 um] Others [< 5mm]	(Fadare et al., 2020)
Pellets	Polyethylene Polymethyl methacrylate Polyester Polyvinyl chloride ...	Microbeads Glitter	Cosmetics [5 – 0.1 mm] [~200 um]	(Miraj et al., 2019) (O'Connor et al., 2019) (Yurtsever, 2019)
Films	Polyethylene Polypropylene Polyamide Polyethylene terephthalate ...	Agricultural mulches Plastic bags Varnish Paint ...	[5 – 1 mm]	(Qi et al., 2020a)
Bioplastics	Polylactic acid Polyglycolic acid Polybutylene succinate Poly(vinyl alcohol) ...	Extrusion leftovers Disposable plastics Agricultural mulches ...	?	(Fojt et al., 2020) (Shruti and Kutralam-Muniasamy, 2019)

Although the first researchers focused on aquatic ecosystems, scientists have recently begun to theorize and study the fate and effects of microplastics in terrestrial environments. It all started with a theoretical question posed in 2012 (Rillig, 2012). Four years later, academic publishers released the first studies about the subject (Huerta Lwanga et al., 2016; Steinmetz et al., 2016). In the years that followed, researchers came to the conclusion that terrestrial ecosystems in general and soils in particular were microplastic sinks. Evidence supporting microplastic pollution in soils began to pile up, building on the first studies that exposed the problem. Five articles on this topic were published in 2018 (Liu et al., 2018; Piehl et al., 2018; Scheurer and Bigalke, 2018; Watteau et al., 2018; Zhang and Liu, 2018). In 2019, the number of scientific reports on microplastics in terrestrial ecosystems exploded, which led the way for scientists to conceptualize microplastic pollution so far. Up to that point, researchers had thought of soils and oceans as microplastic sinks. Nowadays, the scientific community aims to develop a global cycle of (micro)plastics with the aim of emulating what they know about other environmental cycles (Rochman and Hoellein, 2020) (Figure 1.3). This new definition of microplastics, grounded in observations about the ubiquity of plastics in the environment, presents new challenges since there is little evidence on how to close the cycle of microplastics on a global scale (Scheurer and Bigalke, 2018). Hopefully, future questions that seem ambitious today will not take long to answer.

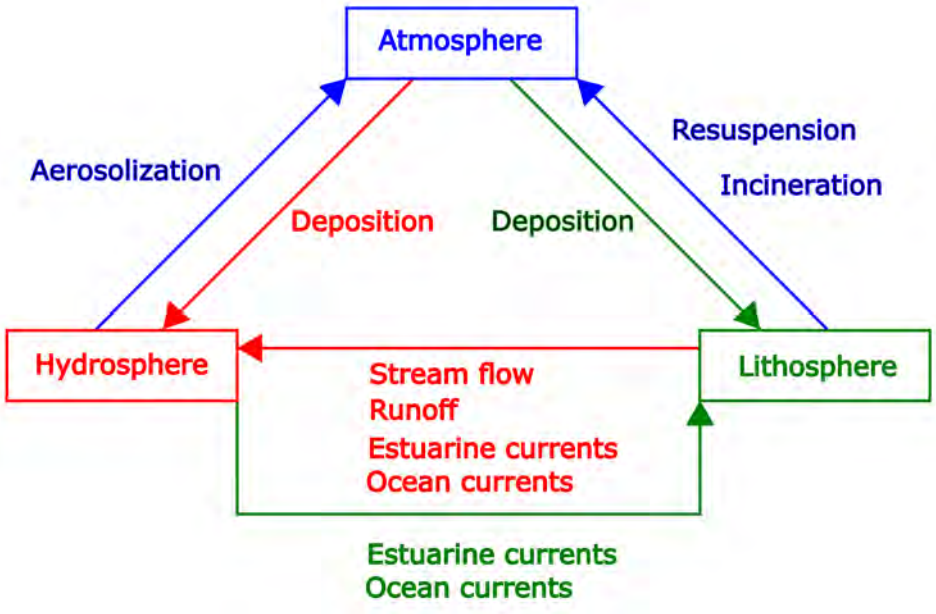

Figure 1.3. *Global cycle of plastics as proposed by Rochman and Hoellein (2020).*

This thesis summarizes my research quest spanning the last four years. I plan to contribute as much as I can to the current debate on the occurrence and ultimate fate of microplastics in the environment. In particular, I hope my research will: (1) provide tools to detect microplastics in soil samples by proposing new methods to extract and identify microplastics in soils (Chapters 1 and 2); (2) present evidence and quantify the occurrence of microplastics in soils and identify potential sources (Chapters 3 and 4).

1.2 Evidence of soil pollution by microplastics: initial studies

The accumulated evidence about plastic pollution in terrestrial ecosystems suggests that plastics buried in soils outnumber by volume their counterparts floating on surface waters. Tudor et al. (2019) estimated that soil pollution by plastics reaches 4 to 23 times the pollution found in aquatic ecosystems. Researchers saw that the accumulation of plastics in soils is steadily increasing since the volume of plastics produced and discarded escalates year after year. Soil scientists have finally started looking at plastic pollution in soil and a limited number of studies have attempted to examine the problem. The Scopus database indexes 22 scientific works that have reported on the ongoing situation (Table 1.2). Studies investigating microplastic occurrence in soils first began in 2018 and have steadily grown in number every year.

The first eclectic studies on the occurrence of microplastics in soils surfaced in 2018. This was a time when experimental designs and hypothesis were guided by what researchers knew about aquatic environments . Within that context, Zhou et al. (2018) carried out one of the most ambitious studies to date in terms of the number of samples examined. The group assessed microplastic occurrence in 129 soils near the coast of Shandong province in China. The authors confirmed what they hypothesized: tourism, aquaculture, construction and other anthropic activities raise the frequency of microplastics in soils. They observed up to 14,700 microplastic particles per kilo of soil (MPs kg^{-1}) in topsoil samples. Also guided by what was known about microplastics in aquatic environments at the time, Scheurer and Bigalke (2018) studied microplastic pollution of floodplain soils in Switzerland. They hypothesized that floods and demographic data would influence microplastic occurrence. Although the authors partially confirmed their hypothesis, an unexpected observation eclipsed their primary finding: they observed microplastics in remote areas without urban settlements or any ongoing upstream human activities (55 to 593 MPs kg^{-1}). Although the study looked at field evidence from a very limited area, scientists considered microplastics in soils and more broadly in all terrestrial ecosystems ubiquitous after this point.

The lack of data on expected concentrations of microplastics in soils was hampering the progress of laboratory-based experiments and motivated the first field-based studies. The

lack of evidence was jeopardizing toxicology studies as researchers set up unrealistic scenarios in their experimental designs (Bläsing and Amelung, 2018). This primary concern drove researchers in Germany to count and identify plastic particles in soil from a farmland in Middle Franconia, South-East Germany (Piehl et al., 2018). The authors chose a farm without former applications of microplastic-containing fertilizers, sludge, or compost and where farmers harvested following conventional agricultural practices. The authors wanted to know whether agricultural activities themselves correlated with microplastic accumulation in soils. They found 0.34 ±0.36 MPs kg^{-1} in the topsoil, and although the authors did not include a control site, their findings spurred new questions. On the one hand, their findings questioned the results of laboratory studies. They confirmed the suspicion that laboratory tests carried out up until that point in time had used unrealistic microplastic concentrations. On the other hand, they pushed soil scientists to wonder what happened in croplands where farmers used technologies that could introduce potential sources of microplastic pollution.

The first data about the occurrence of microplastics in farm soils led to the study of the role of agricultural activities on microplastic pollution. China funded one of the first studies that examined agricultural practices as a source of microplastics. Zhang and Liu (2018) sampled 5 farms near Diana Lake to measure the amount of microplastics in farmlands where farmers regularly applied sewage sludge to improve soil, irrigated with wastewater, and grew crops inside polyethylene greenhouses. Their measurements greatly surpassed the observations of Piehl et al. (2018). Researchers observed between 7,100 and 42,960 MPs kg^{-1} in samples taken at a depth of 0 – 10cm . Moreover, scientists observed that soil aggregates surrounded most microplastics (72%), suggesting that soils played a role as microplastic sinks. The same year, another team of Chinese researchers sampled 40 sites near Shanghai to trace pollution sources and expanded on the evidence collected from Diana Lake (Liu et al., 2018). The authors' findings revealed that plastic mulches, used to improve soil conditions for growing, and sewage sludge applications, used to increase soil organic matter, constituted the major pollution drivers in Shanghai's soils. The authors observed that microplastics measuring less than 1mm were dominant among the plastic fragments they found. Also, researchers observed the highest concentration of microplastics in the topsoil, regardless of the pollution source. Overall, the evidence from both Chinese studies suggested that intensive agricultural activities correlated with terrestrial microplastic pollution.

Although, in general, the scientific community harmonized their observations well and basically agreed on a general cause-effect hypotheses, some observations fell outside of the normal boundaries. In France, researchers observed that the use of municipal compost delivered microplastics to soils (Watteau et al., 2018). The study made antagonistic conclusions. First, the authors did not find evidence of microplastics in the control soils.

Therefore, they suggested that pollution sources caused an accumulation of microplastics locally and that off-site pollutant dispersion rarely occurs. This observation contradicted what Scheurer and Bigalke (2018) proposed after their observations on the Swiss floodplain soils. Second, the authors reported that the soil matrix rarely trapped microplastics within its aggregates, contradicting the observations made by Zhang and Liu (2018) at Diana Lake in China. The emerging evidence and the apparently contradicting observations spurred new research efforts to determine pollution sources (Section 1.3) and to identify the role of soils in the bigger picture of microplastic pollution (Section 1.4).

Table 1.2. *Reverse chronological list of published scientific articles that, as of June 2020, aimed to explicitly quantify microplastics in soils. The table indicates the country of origin (country) of the samples, the number of different sites assessed, the main sources of microplastics observed (source), the extraction and identification method the authors reported, the quantity of particles measured per kilo (mean or range), and the polymers observed.*

Author & Year	Country	Sites	Main source	Extraction method	Identification method	Quantity	Polymers found*
(Zhou et al., 2020a)	China	15	Plastic mulch	Flotation (NaCl & NaI)	Visual sorting** & μ-FTIR	503 ±510	PE PP PET PA
(Zhang et al., 2020c)	China	4	Plastic mulch	Flotation (H_2O)	Visual sorting & μ-FTIR	0 – 800	PE
(Zhang et al., 2020b)	China	3	Sludge	Flotation ($ZnCl_2$)	Visual sorting & μ-FTIR	546 ±46	PE PP PET PB EVA
(Wang et al., 2020b)	China	3	Plastic mulch	Flotation (NaCl)	Visual sorting & Energy spectrometry	2526 – 6070	—
(van den Berg et al., 2020)	Spain	16	Sludge	Flotation (H_2O & NaI)	Visual sorting	5190 ±1930	—
(Huang et al., 2020a)	China	3	Plastic mulch	Flotation (NaI)	Visual sorting & μ-FTIR	1076 – 80	PE
(Feng et al., 2020)	China	17	Agriculture Urban areas	Flotation (NaCl)	Visual sorting & Raman spectroscopy	43 – 52	PE PA PP PS
(Du et al., 2020a)	China	12	Industry	—	TOF-SIMS[1]	—	PA PET PP PVC
(Ding et al., 2020)	China	9	Agriculture	Flotation (NaCl & $CaCl_2$)	Visual sorting[2] & FTIR	1430 – 3410	PS PE PP HDPE PVC PET
(Crossman et al., 2020)	Canada	4	Biosolids	Flotation (—)	Visual sorting & μ-FTIR	541	PE PET PA PP PUR PAN

Author & Year	Country	Sites	Main source	Extraction method	Identification method	Quantity	Polymers found*
(Chen et al., 2020)	China	20	Traffic Domestic waste Agriculture	Flotation ($ZnCl_2$)	Visual sorting & Raman spectroscopy	320 – 12560	PA PP PS PE PVC
(Cattle et al., 2020)	Australia	3	Compost Biosolids Poultry	Wet sieving	Visual sorting	—	—
(Amrutha and Warrier, 2020)	India	5	General assessment	Flotation ($ZnCl_2$)	Visual sorting & FTIR	26 – 205	PE PET PP
(Zhou et al., 2019)	China	24	General assessment	Flotation (NaCl & $ZnCl_2$)	Visual sorting & Raman spectroscopy	2.2e4 – 6.9e5	PE PA PP
(Rezaei et al., 2019)	Iran	11	Wind	Flotation (H_2O)	Visual sorting	67 – 400	—
(Li et al., 2019b)	China	6	Agriculture	Flotation (NaI)	Visual sorting & FTIR	420 – 1290	PE PP
(Zhou et al., 2018)	China	120	Human activities Mariculture Tourism Construction	Flotation (NaCl & NaI)	Visual sorting & Electron microscopy & FTIR	1 – 14713	PE PP PS PUR
(Zhang and Liu, 2018)	China	5	Sludge Agriculture Irrigation with wastewater	Flotation (NaI)	Visual sorting	7100 – 42960	—
(Watteau et al., 2018)	France	1	Municipal compost	Wet sieving	TEM[3] & Py/GC/MS[4]	—	—

Author & Year	Country	Sites	Main source	Extraction method	Identification method	Quantity	Polymers found*
(Scheurer and Bigalke, 2018)	Switzerland	29	Population density	Flotation (NaCl & $CaCl_2$)	FTIR	55 – 593	PE
(Piehl et al., 2018)	Germany	1	Agriculture	Wet sieving	Visual sorting & FTIR	0.34 ±0.36	PE PP PS
(Liu et al., 2018)	China	20	Plastic mulch Sludge	Flotation (NaCl)	Visual sorting & μ-FTIR	78 ±13 63 ±13	PP PE PET

* Polyethylene (PE), polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), polyamide (PA), polybutylene (PB), ethylene vinyl acetate (EVA), high density polyethylene (HDPE), polyvinyl chloride (PVC), and polyurethane (PUR).

** The 'visual sorting' test requires a stereomicroscope to look for microplastics.

[1] Time-of-flight secondary ion mass spectrometry.

[2] Ding et al. (2020) did the visual sorting with a metallographic microscope.

[3] Transmission electronic microscopy.

[4] Pyrolysis coupled to gas chromatography and mass spectrometry.

1.3 Potential sources of microplastics found in soils

To date, scientists have identified several microplastic pollution sources. Most sources emit microplastics to both terrestrial and aquatic environments. This section focuses on sources of pollution for soils, overlooking the source's influence on aquatic environments. Table 1.3 lists all major microplastic sources identified to date.

Table 1.3. *Major microplastic sources identified to date.*

Location	Sources of microplastics
Urban	**Domestic waste**
	Grey water
	Laundry
	Medical applications
	Pharmaceuticals
	Traffic [Transport and recreation]
Industry	**Maintenance and manufacturing**
	Packaging
Agriculture	**Irrigation**
	Plastic mulch
	Plastic covers
	Compost
	Sludge based fertilizers

Human activities are *the* driver of microplastic pollution since plastic production itself constitutes a man-made process (i.e. pristine ecosystems do not have background concentrations of plastics). Urban settlements as well as industrial and agricultural activities are the biggest signs of human interventions. These elements of modern life facilitate the transportation of microplastics to the environment in different ways. Researchers consider the disposal of plastic in unauthorized sites one of the biggest emission pathways of microplastics to the environment (Jambeck et al., 2015). However, urban areas discharge

microplastics through several mechanisms. Amrutha and Warrier (2020) offered a general impression of this discharge. Tracing a river catchment in India, the authors determined how plastic pollution increased downstream of where population density and industrial activities were concentrated. They observed that packaging materials and cloth fibres made up most of the microplastics found (polyethylene and polyester), indicating that the pollution problem was rooted in mismanaged waste and inefficient wastewater treatment facilities. On the role of industrial activities, Du et al. (2020a) reported high microplastic counts in soils near industrial parks and small shops in the suburbs of Baoding City. The authors observed that polyamides (e.g. nylon) were dominant among the plastic fragments they found and linked their occurrence with clothing and shoe manufacturers. The authors related the microplastic pollution with other industrial activities too which they classified as non-ferrous, such as pipe processing and the manufacture of automotive parts and hardware products. According to the authors, these industries used large amounts of polyethylene terephthalate powder. A polymer they found in the soil samples. Yet another effect of urban areas and human activities: traffic was the source of microplastics found in nearby soils. In this regard, Chen et al. (2020) showed how traffic flow affected suburban soils in Wuhan province (China). The authors, who sampled 20 farmlands near the city, claimed that widespread microplastic pollution affected the area. They observed the highest counts of microplastics in soils near the roads (up to 12560 MPs kg^{-1}). However, the authors did not choose to sample agricultural soils. Researchers have long assumed that agricultural practices cause microplastics to accumulate in soils (Nizzetto et al., 2016b).

Research on the role of agriculture as a source of microplastics in terrestrial environments began simply because agricultural activities in general were one of the many human activities to study. After Piehl et al. (2018) published the first study postulating that agricultural activities were a pollution source for microplastics (see section 1.2), other broad environmental assessments supported their findings (Table 1.2). Feng et al. (2020) evaluated soils in the Tibetan Plateau in China and concluded that agricultural practices in general and the use of plastic mulch in particular were some of the main causes for the microplastics found in soils. Ding et al. (2020) came to a similar conclusion after researchers evaluated 9 agricultural sites in Shaanxi, China. The authors argued that the farmers' poor management practices and waste disposal caused the pollution at the study site. Other scientific studies addressed specific microplastic sources of the plastics found in croplands.

Microplastics reach croplands by a range of pathways including unintentional transport within agricultural inputs, such as organic amendments, which play an important role. Section 1.2 discussed only the research of Watteau et al. (2018). The study looked at the occurrence of microplastics in French soils amended with municipal compost. However, the group of organic amendments that could carry microplastics to soils was composed of more than just one product (Table 1.2). Cattle et al. (2020) evaluated the accumulation of

microplastics in 3 experimental plots treated with biosolids, compost, and poultry manure. The authors found that microplastics accumulated in soil regardless of the organic amendment used, and that translocation to deeper soil layers rarely occurs. The assessment of 4 sites amended with biosolids in Canada reinforced the hypothesis that biosolids transport significant amounts of microplastics to soils (Crossman et al., 2020). The Canadian assessment revealed that fibres get entangled around soil aggregates, where they remain fixed to the soil matrix. Two other studies focused on the cause-effect relationship between the agricultural use of sewage sludge and sludge-derived products (pellets, fertilizers, etc) and microplastic pollution. Altogether, both studies reported the occurrence of microplastics at 3 sites in China (Zhang et al., 2020b) and at 16 sites in Spain (van den Berg et al., 2020). Both studies concluded that the application of sewage sludge to farmlands caused the unintentional accumulation of microplastics in agricultural soils.

Besides the unintentional introduction of microplastics to soils by agricultural practices, farmers might introduce plastics intentionally to their croplands by using plastics to improve crop productivity. Farmers often use plastic mulch to enhance water retention or modify soil temperature. Plastic residues remain in the fields after farmers remove plastic mulches between seasons, thus the residues slowly accumulate in the soil over time. Chinese science funding agencies decided to place special attention on this potential entryway for microplastics to soils (Table 1.2). Four study cases from China reported that tearing and burying plastic mulches resulted in the accumulation of microplastics in the soil profile (Huang et al., 2020a; Wang et al., 2020b; Zhang et al., 2020c; Zhou et al., 2020a). The four studies presented significant observations that have raised environmental concerns about the fate of microplastics once they enter the soil. Section 1.4 summarizes the concerns raised by these studies The off-site transport processes for microplastics and the relocation of these plastics within the soil profile are some points that worry researchers.

In addition to exposing some of the potential sources of microplastics, the evidence accumulated so far only partially clarifies the mechanisms by which microplastics are relocated within terrestrial ecosystems. Although it is true that agricultural activities and urban areas are the main sources of plastic pollution for nearby sites, different transport agents play a role in relocating and spreading microplastics off-site. Water can transport microplastics from their sources to sinks either as suspended particles or bed loads in superficial water courses (Amrutha and Warrier, 2020). Moreover, water can carry microplastics to floodplain soils (Scheurer and Bigalke, 2018) and coastal plains (Zhou et al., 2018). Water can also relocate microplastics within the soil. Evidence suggests that irrigation drags microplastics through soils horizontally (Zhang et al., 2020c) and vertically, burying them in deeper soil layers under the topsoil (Wang et al., 2020b; Yu et al., 2019). Even more, water carries microplastics to soil indirectly since water transports domestic wastewater containing plastic fibres, the most common microplastic shape found in soils

(Kumar et al., 2020), to wastewater treatment plants. Once here, the sludge traps a large proportion of the microfibres (Ziajahromi et al., 2017), which might ultimately end up in croplands due to sludge disposal (Chapter 4). Wind also acts as a transport agent for microplastics. Wind and atmospheric deposition transport microplastics from hotspots, such as cities (Liu et al., 2019a), towards remote areas without direct or ongoing human activities (Rezaei et al., 2019; Zhang et al., 2019). As discussed, water, waves, and wind might transport microplastics from sources to sinks. Ice is the only erosion agent that scientists have not found to be a mode of transport for microplastics. This could soon change as researchers gather evidence concerning the presence of microplastics in snow and glaciers in Europe (Ambrosini et al., 2019; Bergmann et al., 2019). Most likely, it won't be long before scientists can support the conjecture illustrated in Figure 1.3 about the global cycle of microplastics with cold hard facts (Rochman and Hoellein, 2020).

Although studies so far have shed light on the factors that favour movement or deposition of microplastics in terrestrial environments, the lack of study cases impedes a thorough comprehension of the global scenario. Scientists and policymakers both need evidence to validate early observations for different environmental conditions. More data from study cases should be integrated with laboratory insights to understand the underlying processes that shape microplastic dynamics in terrestrial ecosystems. Therefore, studies should gather and report more temporal (Chapter 4's motivation) and spatial (Chapter 5's motivation) data. On the lack of temporal data, only a couple of studies have reported temporal accumulation patterns. One study looked at the impact of 24 years of uninterrupted plastic mulch farming (Huang et al., 2020a), while the other focused on the effects of long-term sludge applications (van den Berg et al., 2020).This raises the question: to what extent would these findings change under different management practices? Both of the studies in question evaluated only agricultural soils. On the lack of spatial data, only a few studies have studied evidence of plastic pollution. Even then, from the 340 sites worldwide, 80% were in China (Table 1.2). Moreover, evidence of plastic pollution in non-agricultural soils is almost completely lacking. After reviewing all of these facts, there are blatant knowledge gaps that scientific studies should fill before policy-makers can design successful prevention and mitigation strategies (Stubenrauch and Ekardt, 2020). This thesis attempts to contribute to the scientific corpus that will fill the knowledge gaps that currently hamper environmental protection.

1.4 Environmental concerns related to the accumulation of plastics in soils

The previous sections summarize the evidence concerning the accumulation of microplastics in soils and the transport process that deposit and relocate these pollutants

in terrestrial ecosystems. This section elaborates on the threats that the accumulation of microplastics in soils pose to the environment as a whole as well as the detrimental effects that microplastics might cause to soil organisms.

Plastics in soils pose a threat to the environment since soils do not hold on to plastic particles permanently. Instead, soils accumulate plastics coming from point sources and eventually release them, thus ultimately acting as a diffuse pollution source. Zhang et al. (2020c) illustrated the case in their study on the drawbacks of using plastic mulch. The authors pointed out that inevitably, plastic mulches tear when farmers remove them from farmlands after the cropping cycle, leaving behind small low-density polyethylene fragments to accumulate in soils (methodological limitations constrained the authors' observations to the 5000 – 50µm size range). These plastic fragments age and deteriorate in soils and become microplastics. At a later stage, surface runoff carries around 96% of these microplastics off-site. The authors concluded that by these means, the plastic mulch ultimately becomes a source of microplastics in aquatic environments. *In situ* studies on off-site movements of microplastic particles have failed to support or contradict the findings of Zhang et al. (2020c). However, one of the few studies on the accumulation of microplastics in agricultural soils reported that surface irrigation decreased the counts of microplastics in the topsoil (Wang et al., 2020b). This observation supports the hypothesis that microplastics are transported off-site by runoff water. Beyond off-site transport, Zhang et al. (2020c) noticed another facet of *in situ* transport processes. The authors discovered that infiltration transports 4% of microplastics down into the soil profile, usually when the size of the plastics are under 100µm. Their evidence suggested that microplastics move downwards through the soil pore space only within soil microaggregates. If true, this fact would imply that aging and aggregation with soil minerals and organic matter trigger the downward mobility of microplastics. The authors concluded that there was a need for more studies to evaluate the downward mobility of microplastics. However, researchers warned that laboratory studies using primary microplastics as opposed to aged particles or those entangled within soil aggregates might offer the wrong insights.

Laboratory studies that assess the mobility of microplastics moving from the topsoil to deeper soil layers support the field observations that suggest organic matter and microplastics move together through soil's pore space. Using marked microplastics, Keller et al. (2020) tested how microplastic fibres from sewage sludge migrate through a column filled with 1mm glass pearls (polyester fibres of Ø = 30µm and 510 ±12µm) . The authors observed that more than 95% of the fibres remained attached to the sludge and stayed at the top of the column. The observation correlates with what Zhang et al. (2020c) described for plastic mulch where 96% of microplastics remained in the topsoil. Keller et al. (2020) went further, however. These researchers observed that the mobile organic fraction of sludge co-transported nanoplastic particles (180nm) along the glass pearl column. As a

result, half of the nanoplastics in the sludge percolated after one simulated rain event. Other studies have indicated that the vertical movement of microplastics in soils constitutes an environmental threat. O'Connor et al. (2019) used polyethylene microbeads (Ø = 180 – 500µm) to study the percolation of microplastics in sand columns. Using their observations, they estimated a penetration rate for microplastics of more than 5m after 100 years of dry-wet cycles for soils near Beijing. Along the same lines, using larger microplastic particles (polyethylene fragments of Ø = 250 – 1000µm) and similar soil columns, Yu et al. (2019) observed that the percolation of microplastics increased with the increasing number of macropores. Thus, soil fauna could accelerate percolation by digging macropores and dragging down microplastic particles (Maaß et al., 2017; Rillig et al., 2017b; Yu et al., 2019). The evidence indicates that soils act as an unsteady sink for microplastics from which erosion agents, such as water and wind (Section 1.3), could transport microplastics that would pollute other (remote) ecosystems.

Up to this point, I have presented soils as one of the pitstops that microplastics make as they cycle through different ecosystems. Keeping this in mind, what detrimental effects do microplastics residing in soils cause on soils and soil biota? Field evidence suggests that microplastics measuring less than 50µm alter the soil pore space since they act as enveloping or nucleating agents for the organic matter and mineral fraction in soils. The formed microaggregates clog pores, reducing network connectivity, water percolation, and gas exchange (Cattle et al., 2020). Laboratory experiments support the observations concerning the effects of microplastics on soil physical properties. For example, Qi et al. (2020b) revealed that 250 to 500µm polyethylene and bioplastic fragments reduced a soil's water holding capacity. Moreover, microplastics adsorbed other pollutants onto their surface: polyethylene, polypropylene, and polyacrylate particles up to 200µm in size built up hotspots for heavy metals within the soil profile (Zhou et al., 2019), and polyethylene and polyethylene-vinyl acetate films of any size hyperaccumulated agrochemicals (Ramos et al., 2015; Yang et al., 2019b). Concerning the effects of microplastics on soil biota, microplastics can affect soil biota directly through ingestion. Researchers have observed this effect primarily in earthworms whose intestines malfunction with the ingestion of polystyrene particles as small as 1µm (Cao et al., 2017; Jiang et al., 2020). For these animals, the detrimental effects are amplified whenever microplastics carry other pollutants into their guts, such as chlorpyrifos or cadmium (Rodríguez-Seijo et al., 2019; Zhou et al., 2020b). These worries now extend beyond soils, as new studies have revealed the absorption of nanoplastic particles by plants (Li et al., 2020c; Li et al., 2019a) and other movements of microplastics through the food chain (Huerta Lwanga et al., 2017b), suggesting the threat of bioamplification.

In summary, the accumulation of microplastics in soils concerns environmental scientists since it poses a threat to soil's biota and functions and since soils, once polluted, could

redistribute microplastics to other remote ecosystems. Although evidence about plastic toxicology and off-site transport has emerged, limited studies have evaluated real-life scenarios (see Section 1.2). The lack of field studies might be related to the lack of standards and appropriate methods to address the problem of counting and identifying microplastics in complex organic matrices. The next Section (1.5) elaborates on these analytical challenges.

1.5 On the challenges of extracting and identifying microplastics in soils

Researchers have struggled to find an appropriate method to identify and quantify microplastics in soils, sludge, and compost since they first began to study microplastics in terrestrial ecosystems (Zhang et al., 2020a). Microplastics pose an analytical challenge since the term aggregates a group of man-made materials that exhibit different properties; from physical, such as density, to chemical, such as resistance to acids (Barcelo, 2020). Although the differences in physical and chemical properties could help to identify different polymers, they force analysts to perform complex sequential steps to identify them all. Polymer identification is not the only analytical challenge that researchers must face. The quantification and extraction of microplastic particles from samples also puzzle analysts (Bläsing and Amelung, 2018). Scientists have proposed alternative analytical methods that do not require extracting microplastics before analysis in order to overcome some of the difficulties they face. However, these alternative methods have their own limitations to quantify or classify the polymers.

Researchers have leaned mostly on spectroscopy or visual sorting to assess soil microplastic pollution for field studies they have reported so far (Table 1.2) (Figure 1.4). Visual sorting with the aid of stereomicroscopes allows scientists to determine the number of microplastics in a sample. By assuming average densities, the analyst might estimate the mass of the observed microplastics. The method offers a simple procedure to assess microplastic pollution of soils. However, misclassifications introduce biases into the results (~7%) and the calculated microplastic mass stands as a mere approximation (Horton et al., 2017). To tackle this problem, Zhang et al. (2018) proposed to melt suspicious particles to confirm their plastic nature. They tested the approach only with LDPE, and further development of the method lacks till today. In any case, visual sorting will never overcome its fundamental flaw: it does not provide information about the particles' polymer type. For this reason, researchers usually implement additional analytical steps to identify polymers when they expect samples with multiple polymer types. The analyst's toolbox includes typically Furrier Transformed Infrared (FTIR) instruments, Raman microscopes, or other analytical techniques based on spectroscopy. However, instrumental analyses by FTIR or

Raman spectroscopy offer additional unresolved challenges for sample pre-processing (Löder and Gerdts, 2015) and post-processing (Chabuka and Kalivas, 2020). For example, software limitations and the lack of comprehensive reference libraries jeopardize accurate polymer detection and identification (Primpke et al., 2019a; Primpke et al., 2018). This is especially true for the case of bioplastics (Fojt et al., 2020). Moreover, FTIR and Raman analyses constitute slow and tedious processes that undermine research efforts and progress (Jany et al., 2020). Despite all the drawbacks, together visual sorting and instrumental classification through spectroscopy offer the best practical solution for environmental assessments to date.

	Identification or quantification	Extraction	Limitations
Common methods	• Visual sorting • Spectroscopy: [Raman FTIR μ-FTIR]	• Flotation [H_2O $NaCl_2$ NaI $ZnCl_2$] • Wet sieving	• Quantity only • Lack of libraries & type only
Complementary techniques	• Spectroradiometry • TED-GC-MS[1] • Py-GC-MS[2] • TOF-SI-MS[3]	No extraction	• High LOD • Type only • Small sample size • Type only

Figure 1.4. *Analytical methods researchers have used to extract and identify microplastics in soil samples from field studies.*

Methods based on spectroscopy or microscopy techniques require prior polymer extraction from the sample matrix. Mimicking the method water scientists built to extract polymers from sediment and sand beach samples, extraction procedures rely mostly on density separation. Density separation ranks first as the method researchers used to extract microplastics from bulk samples in all soil assessments done so far, with 20 out of 23 articles reporting this methodology (Table 1.2). The 3 studies that deviated from this trend extracted microplastic particles from soil samples using wet sieving. In other words, researchers have generally not employed any alternative to density separation in any of the soil assessments done so far. This tacitly counteracts the common claim that says the methods we use lack standardization (Wu et al., 2020a). Scientists might not have

standardized a method yet, but at least some *de facto* steps have begun to take root in today's workflows.

Even though current studies have often reported using density separation within the material and methods section of their papers, this method might undermine analytical results. For instance, the construction of the extraction devises and the samples with a high organic matter content (>4%) jeopardize the method's performance. Buoyant particles stick to the walls of the tubes used in the extraction devises thus diminishing recovery (Karlsson et al., 2017). Custom-made permanent flow devices that isolate buoyant particles by solvent overflow prevent this drawback (Liu et al., 2019c). High organic matter content hampers recovery first by clogging the filters that retain the microplastics and later by eclipsing microplastics during microscopy (Fuller and Gautam, 2016; Mahon et al., 2017). Enzymes or acids can digest or oxidize organic matter. However, enzymes might not digest organic matter completely (Löder and Gerdts, 2015) and acids might damage the microplastics (Hurley et al., 2018). Despite the efforts to improve density separation and because of its flaws, every new article that contributes evidence about soil microplastic pollution presents a slightly modified version of the method. This fact underlines the need for standardization and alternatives to density separation.

The flaws of density separation have pushed scientists to research alternative methods to extract microplastics from complex organic matrices. Interestingly, a discontinued proposal to exploit the electrostatic behaviour of microplastics offered a completely different approach (Felsing et al., 2018). Unfortunately, the proposal was overlooked by subsequent works. Another approach explored in recent publications relied on oils to perform the extraction, profiting from plastic's lipophilic properties (Mani et al., 2019; Scopetani et al., 2020). However, disregarding how good they might sound, all alternatives to density separation are still proof of concepts and researchers should escalate them prior to their inclusion in the analyst's toolbox. Anyhow, regardless of the method they choose, researchers and analysts will face the intrinsic problem of adding additional steps to an analytical workflow; extraction, here intended as an avoidable step, will introduce additional and unnecessary uncertainty into the results. Therefore, whenever possible, the analyst should choose methods that do not require extraction.

Scientists have explored a few alternative methods that do not require the extraction of microplastics from the bulk soil sample before analysis to quantify or identify their presence. For example, optical methods based on differences in polymer reflection properties offer a fast solution to discriminate whether a soil sample contains microplastics or not (Ng et al., 2020b; Paul et al., 2019). Following this approach, researchers can assess soil microplastic pollution in field conditions with portable near infrared spectroradiometers (Qiu et al., 2020a). However, spectroradiometer measurements can

only categorize samples as plastic positives or negatives. They do not quantify microplastic pollution. Other alternative methods pose different problems. Technicians can identify plastic polymers with great precision and accuracy within soil or sludge samples via thermal desorption–gas chromatography–mass spectrometry (TED-GC-MS). However, this method does not quantify the amount of the different polymers present (Dümichen et al., 2015; Dümichen et al., 2017b). Similarly, time-of-flight secondary ion mass spectrometry detects plastics to a nanometric scale identifying their composition and yet does not quantify polymers (Du et al., 2020b). Thus, alternative soil tests to measure microplastic content do not provide simple solutions to detect, classify, and quantify the polymers in soil samples in one go. Their deficiencies have two main consequences. First, methods based on spectroscopy or microscopy techniques predominate as research methods. Second, the development of alternative analytical methods blossoms as research topic.

1.6 Hypothesis

This PhD thesis aims to add to the growing body of evidence that identifies and clarifies the sources and dynamics of microplastics in terrestrial ecosystems. It intends to shed light on the occurrence of microplastics across different land uses and to reveal major pollution sources. To attempt this endeavour, in the first two chapters I discuss new insights on analytical methods to quantify and identify different microplastic polymers in soil samples (Chapters 2 and 3). In later chapters, I use the proposed methods to collect evidence concerning human influence on soil microplastic pollution. The two study cases this thesis examines in the last two chapters describe microplastic pollution in soils exposed to: 1) sludge application (Chapter 4), and; 2) different land uses at a regional scale (Chapter 5). Samples came from Chile, from a region with a warm temperate climate and mixed land usage which also happens to hold one of the most highly populated Latin American cities. The selected study location increased our understanding of the problem because: 1) it stands as an example of how human activities in upper-middle income countries contribute to the global problem of plastic pollution, and 2) since the weather conditions recreate Mediterranean ecosystems, the evidence can be extrapolated to other world locations that are underrepresented in current scientific literature.

This PhD thesis tests the following hypothesis:

- Visible to near infrared (vis-NIR) spectroradiometers can predict the concentration of selected polymers in soil samples without pre-processing the samples (Chapter 2).

- A spectral angle mapper algorithm and process parallelization can optimize current library search algorithms and software to post-process FTIR readings and find microplastics in soil samples (Chapter 3).
- Sludge disposal in agricultural fields transport microplastics to soils and successive applications result in the higher accumulation of microplastics in croplands (Chapter 4).
- Intensive agricultural practices and dry or wet deposition as a result of offsite transport from roads and urban areas extend the problem of microplastic pollution of soils beyond the limits of croplands, polluting other soils (Chapter 5).

1.7 Outline of this thesis

This PhD thesis comprises 6 chapters. Chapters 2 to 5 present the works including the evidence and the discussion that examine the hypotheses introduced in Section 1.6. They constitute complete scientific works that can be read independently. For this reason, people reading this thesis cover-to-cover may notice some redundancies in the chapters.

Chapter 1 offers a general introduction to the thesis. It presents current evidence about the occurrence of microplastics in soils (Section 1.2), potential sources and entryways (Section 1.3), environmental concerns and risks (Section 1.4), and analytical challenges (Section 1.5).

Chapter 2 explores the possibility of using a vis-NIR analysis technique as a novel, fast, and scalable method to identify and quantify microplastics in soil samples. It aims to predict the concentration of low-density polyethylene (LDPE), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) in artificial soils using a portable spectroradiometer and avoiding extraction steps.

Chapter 3 summarizes the functionalities of new software that implements parallelization and a spectral angle mapper algorithm to optimize current postprocessing tools that analyse FTIR data on microplastics in soils. It refers to a software published through the R-CRAN repository (the Comprehensive R Archive Network).

Chapter 4 presents a study case in Chile that addresses the question: Do microplastics accumulate in agricultural soils as a result of sewage sludge applications? It delivers evidence on the microplastic pollution of soils due to sewage sludge applications and it evaluates the impact of repeated sewage sludge applications on croplands.

Chapter 5 presents a study case in Chile that provides evidence on the occurrence of microplastics across different land uses and their correlation with other indicators of soil

anthropogenic pressure. It shows data on the presence of microplastics in the topsoil under different land uses at the regional level in Chile's central valley.

Chapter 6 summarizes the major conclusions of this research and discusses the implications of its findings. The chapter ends by suggesting directions for future research work.

1.8 Study area (chapters 4 and 5)

Chile lies in the southern hemisphere of the American continent. A small region located in the northern part of Chile's Central Valley serves as the backdrop of Chapters 4 and 5 (Figure 1.5.). Although Chapters 4 and 5 relate to the same geographical area, they tackle different knowledge gaps. Chapter 5 assesses soil microplastic pollution in the broad context that Chile's Región Metropolitana offers. Chapter 4, however, studies a particular situation within the region: sludge disposal. Please refer to Chapters 4 and 5 for detailed descriptions of the study area and its connections to the hypothesis of this thesis (section 4.2.1 and section 5.2.1).

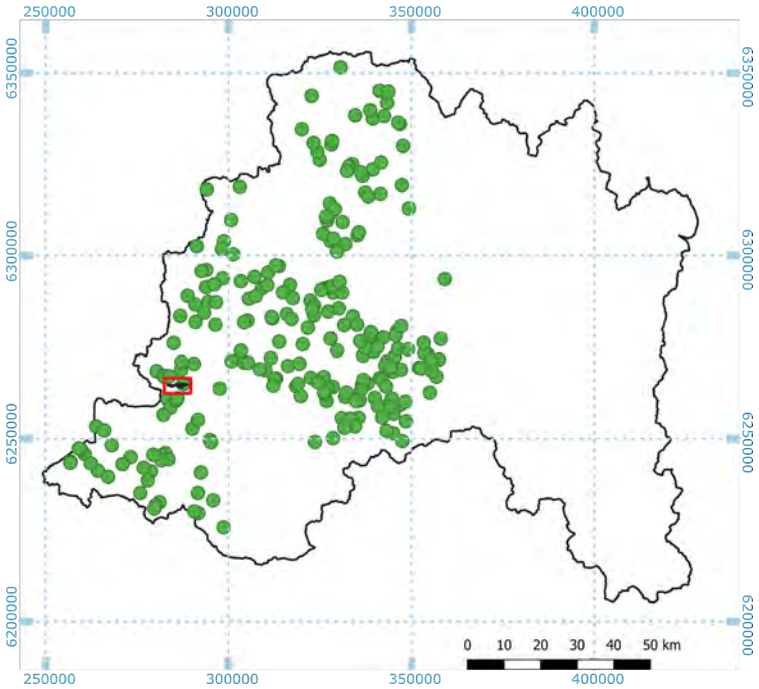

Figure 1.5.*Chile's Regi'on Metropolitana. The red square shows the location where the fields of Chapter 4 are, and the green dots show the sampling points of Chapter 5.*

Chapter 2. Predicting soil microplastic concentration using vis-NIR spectroscopy

Microplastic accumulation in soil may have a detrimental impact on soil biota. The lack of standardized methods to identify and quantify microplastics in soils is an obstacle to research. Existing techniques are time-consuming and field data are seldom collected. To tackle the problem, we explored the possibilities of using a portable spectroradiometer working in the near infrared range (350-2500nm) to rapidly assess microplastic concentrations in soils without extraction. Four sets of artificially polluted soil samples were prepared. Three sets had only one polymer polluting the soil (low-density polyethylene (LDPE), polyethylene terephthalate (PET), or polyvinyl chloride (PVC)). The fourth set contained random amounts of the three polymers (Mix). The concentrations of microplastics were regressed on the reflectance observed for each of the 2150 wavelengths registered by the instrument, using a Bayesian approach. For a measurement range between 1 and 100 g kg^{-1}, results showed a root-mean-squared-deviation (RMSD) of 8, 18, and 10 g kg^{-1} for LDPE, PET, and PVC. The Mix treatment presented an RMSD of 8, 10, and 5 g kg^{-1} for LDPE, PET, and PVC. The repeatability of the proposed method was 0.2 – 8.4, 0.1 – 5.1, and 0.1 – 9.0 g kg^{-1} for LDPE, PET, and PVC, respectively. Overall, our results suggest that vis-NIR techniques are suitable to identify and quantify LDPE, PET, and PVC microplastics in soil samples, with a 10 g kg^{-1} accuracy and a detection limit ≈ 15 g kg^{-1}. The method proposed is different than other approaches since it is faster because it avoids extraction steps and can directly quantify the amount of plastic in a sample. Nevertheless, it seems to be useful only for pollution hotspots.

Based on:

Corradini, F, H Bartholomeus, E Huerta-Lwanga, H Gertsen, V Geissen. 2019. Predicting soil microplastic concentration using vis-NIR spectroscopy. Science of the Total Environment 650:22-932.

2.1 Introduction

While in marine environments plastic debris and microplastics (particles < 5mm) have long been considered pollutants and thus have been studied broadly since the early 1970s (Carpenter and Smith Jr, 1972), less information has been collected about soil as a microplastic sink (Bläsing and Amelung, 2018). Yet, these particles have proven to be ubiquitous in terrestrial ecosystems (de Souza Machado et al., 2018a). Research on the topic has only begun over the last few years and is mainly focused on the impacts of microplastics on soil biota(Huerta Lwanga et al., 2016; Huerta Lwanga et al., 2017b; Maaß et al., 2017; Rillig et al., 2017b). Researchers have struggled to identify and quantify microplastics in soil samples due to the lack of standardized methods (de Souza Machado et al., 2018a) since Rilling put forth the idea in 2012 (Rillig, 2012).

Although there are some methodologies available to detect and qualify microplastic concentrations in sediments and water, such as Raman and Fourier-transform infrared spectroscopy (FT-IR) (Crawford and Quinn, 2017b), these methods have not been standardized for complex matrices such as soils and require appropriate sample preparation (Crawford and Quinn, 2017a). Other alternatives are visual sorting, which is a simpler and cheaper option (Lots et al., 2017), and pyrolysis–gas chromatography–mass spectrometry (Pyr-GC-MS). While the former is subject to bias from human-errors and precision limitations (Ziajahromi et al., 2017), Pyr-GC-MS presents some drawbacks since it needs adequate concentration or separation steps, which could limit the analysis of large quantities of microplastics (Crawford and Quinn, 2017b; Löder and Gerdts, 2015). To tackle the problem, new chromatographic approaches that allow for bigger sample loads have been proposed, such as thermal desorption GC-MS (TED-GC-MS) (Dümichen et al., 2015; Dümichen et al., 2017b).

All of the proposed methods require sample preparation steps with the density separation approach being the most broadly used to isolate microplastics from bulk samples. Flotation methods similar to the one proposed by Zubris and Richards (2005) and later by Zhang et al. (2018) are commonly used. These methods use density to differentiate between plastic particles and the particles naturally found in soil. This could include analytical steps needed to accelerate particle separation such as the use of saturated salt solutions and/or centrifugation (Duis and Coors, 2016; Pita and Castilho, 2017; Zhang et al., 2018).

Nevertheless, studies regarding the microplastic pollution of soil have been performed under laboratory conditions and have focused on the effects of microplastics on soil biota. These studies have seldom reported recovery rates or quality control procedures. Moreover, researchers commonly used only two or three plastic polymers with densities low enough to assure particle flotation since the quantification of soil microplastic content

was not the core of their research (e.g. Huerta Lwanga et al., 2017a; Maaß et al., 2017; Ramos et al., 2015). In this regard, Ziajahromi et al. (2017) pointed out that standard sampling and processing methods for microplastics in organic-rich samples are still insufficient, highlighting the importance of thorough characterization of microplastics to avoid false detection and study biases. Pressurised fluid extraction (Fuller and Gautam, 2016) and elutriation (Claessens et al., 2013; Mahon et al., 2017) are the current alternatives but they are not free from interference.

Although straightforward methods for microplastic quantification take advantage of plastics' spectroscopic properties, such as FT-IR and Raman spectroscopy, the use of visible (vis), near-infrared (NIR), and shortwave infrared (SWIR) equipment to detect and characterize microplastics have received less attention. A vis-NIR spectrometer measures the amount of light that is reflected from a surface within the wavelength range of 350 to 2500 nm, giving a reflected percentage for each wavelength. This information can be correlated with the chemical composition of the sample and thus it allows for predicting the composition of new sample sets. As vis-NIR techniques have been useful to examine elemental composition directly on soil bulk samples (Conforti et al., 2018; Gandariasbeitia et al., 2017; Viscarra Rossel et al., 2016), it could be possible to use these methods to avoid or reduce sample preparation steps, tackling one of the current problems in microplastic detection. Moreover, as the vis-NIR spectra is correlated with the chemical composition of the sample, this method could be useful in microplastic quantification.

There are public vis-NIR spectra datasets showing that different plastic polymers commonly found in the environment have different spectral signatures (reflectance along a wavelength range) (Garaba and Dierssen, 2018). Therefore, these plastic polymers might be identifiable by using vis-NIR spectrometric techniques. Nonetheless, to our knowledge, there have been no previous studies using these techniques to evaluate microplastics in soil samples. Our expectation for this work was to explore the possibility of using a vis-NIR analysis technique as a novel, fast, and scalable method to identify and quantify the amount of microplastics in soil. Consequently, the aim of this work was to predict the microplastic concentration (low-density polyethylene (LDPE), polyethylene terephthalate (PET), and polyvinyl chloride (PVC)) of soil samples using a portable spectroradiometer while avoiding extraction steps. We did so by making custom-made artificially polluted soil samples to evaluate the spectral characteristics of LDPE, PET, and PVC and their interaction with soil. Subsequently, we evaluated the quantification effectiveness of the device by training a multilinear model, regressing the quantity of the added plastic on the observed reflectance, to predict the microplastic content of a given sample. Lastly, the method we propose was used to predict the concentration of a specific plastic polymer in samples polluted with a mixture of LDPE, PET, and PVC, assessing the method's qualification capability.

2.2 Material and methods

2.2.1 Experimental design

To examine the concept of using vis-NIR techniques to quantify microplastics in soil, a spectroradiometer was used to record the reflectance spectra (section 2.2.4) of laboratory-made polluted soil samples (section 2.2.3). Four treatments were used to evaluate the performance of the technique, each including a different plastic polymer (Table 2.1). While the first three treatments included only one plastic polymer (LDPE, PET, and PVC), the fourth had concentrations of each of the three polymers used in the former treatments (Mix). Each treatment included a training and a testing dataset (section 2.2.3) ranging in concentrations between 1 and 100 g kg^{-1} (0.1 and 10% by weight) for LDPE, PET, and PVC, and 1 and 80 g kg^{-1} (0.1 and 8.0%) for the Mix. The training set was used to train a linear model by regressing the known plastic concentration on the recorded reflectance (section 2.2.5.2). The testing set was used to evaluate the accuracy of the model when predicting soil plastic concentrations for a new set (section 2.2.5.3 and 2.2.5.4). The testing data sets of the Mix treatment considered 3 measurement repetitions in order to evaluate the method repeatability (section 2.2.5.4). An overview of the experimental design is shown in Fig. 2.1.

Table 2.1 *Plastic polymers used in the different treatments, and treatments designation.*

Treatment	Plastic polymer	Plastic colour	Source material	Production method	Size (mm)
LDPE	**Low-density polyethylene**	**White**	**Pellets**	**Freezing, Milling**	**1 to 0.5**
PET	**Polyethylene terephthalate**	**Various**	**Food packaging**	**Chopping, Grinding**	**1 to 0.5**
PVC	**Polyvinyl chloride**	**Grey**	**Board**	**Filing, Rasping**	**1 to 0.5**
Mix	**All three**	**Various**	**All three**	**All three**	**1 to 0.5**

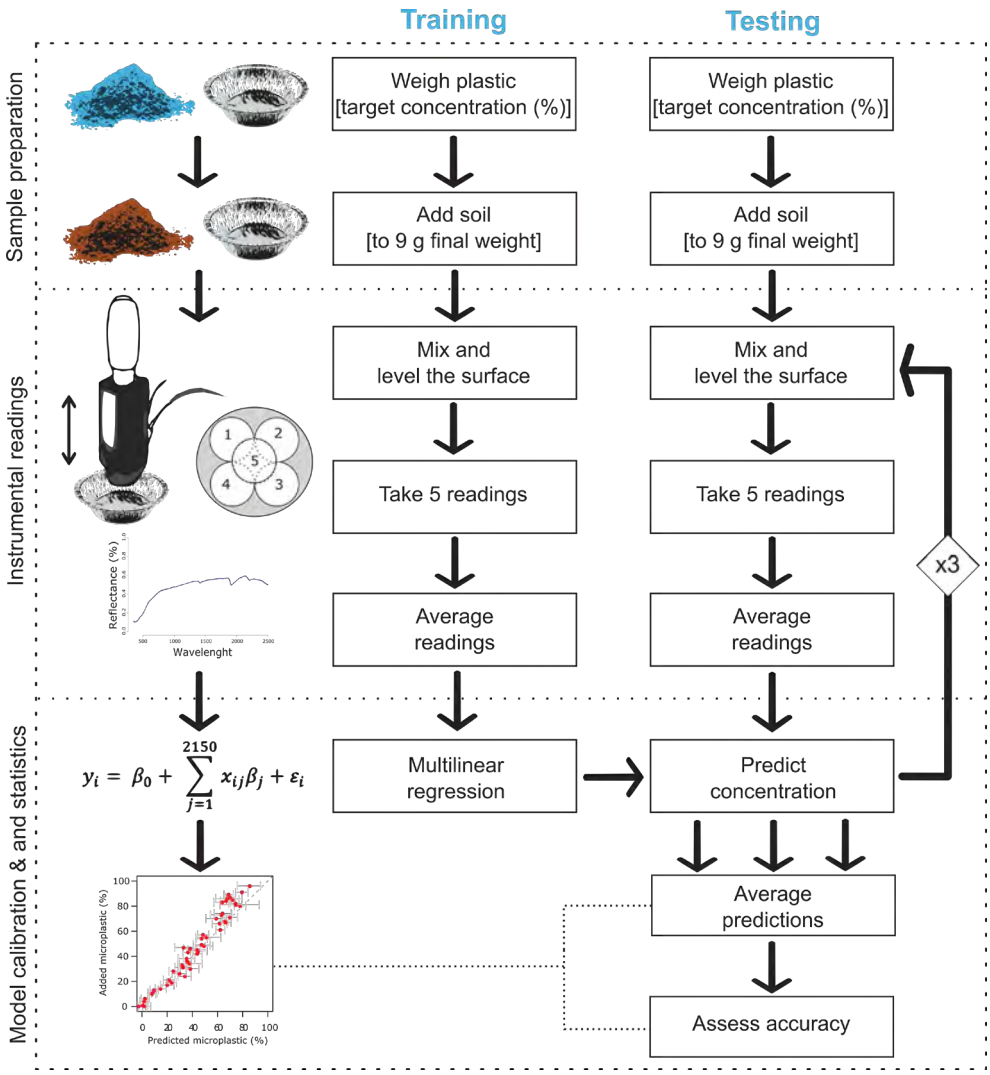

Figure 2.1 *Analytical steps within a treatment. A defined microplastic weight was added to an aluminium pot according to the desired concentration and the total weight of the sample was set to 9 g by adding soil to the pot. The polluted sample was mixed and levelled to get an homogeneous surface and five readings were taken from different positions following a quincunx. The final measurement stood as the average of the five readings. The spectra recorded for the training data was used to calibrate a multilinear model by regressing the added amount of plastic on the observed reflectance. Later, the model was used to predict the concentration of microplastics of the testing set samples. Three predictions from three spectral replicates were made for each sample within the testing set to report the final result, which was used to assess the prediction accuracy.*

2.2.2 Soil and microplastic preparation

Loess top-soil (0 - 30 cm) collected in Limburg, The Netherlands was used (50% of sand, 50% of silt and clay, and 3% of organic matter). The soil was oven-dried at 40˚C and sieved at < 2 mm. Low-density polyethylene (LDPE), polyethylene terephthalate (PET), and polyvinyl chloride (PVC) particles were used as plastic pollutants. Polyethylene particles were obtained by freezing and milling low-density polyethylene pellets (SABIC® LDPE). The particles were classified by size after sieving the plastic powder. The fraction size that ranged from 1 to 0.5 mm was used. Polyethylene terephthalate particles were produced by chopping and grinding food and liquid PET containers with a modified paper crusher, passing the plastic residues through the machine several times. The particles were sieved to recover the 1 to 0.5 mm fraction. Polyvinyl chloride particles were made by filing and rasping a PVC board using a flat file and a rectangular sectioned rasp. The PVC chips were sieved and the 1 to 0.5 mm fraction stored. Once made, aluminium pots of 6 cm in diameter were used to hold the samples (section 2.2.3).

2.2.3 Treatments and sample preparation

Four treatments of artificially polluted soil samples were made (Table 2.1). Each treatment comprised a training set to calibrate a predictive model (section 2.2.5.2) and a testing set to evaluate the model quantification and qualification effectiveness (section 2.2.5.3 and 2.2.5.4). The treatments LDPE, PET, and PVC comprised a set of 150 soil samples polluted with the corresponding plastic polymer plus ten pure soil controls ranging in concentrations between 0 and 100 g microplastics kg^{-1} (0.0 and 10% by weight). The Mix treatment comprised twenty samples polluted with all three polymers used (PE, PET, and PVC) ranging in concentrations between 0 and 80 g microplastics kg-1 (0.0 and 8.0% by weight).

2.2.3.1 Training sets

Treatments LDPE, PET, and PVC had each a training set of 100 samples plus five of the pure soil controls. For each treatment, the samples were made by adding cumulative plastic weights to the aluminium pots starting from 9 mg to 900 mg, increasing the amount of plastic added by 9 mg between each pot (±0.001g model XL-410 Denver Instruments, NY). Later, soil was added to the pots for a total weight of 9 g. By this means, the obtained 100 samples presented a discrete microplastic concentration range from 1 to 100 g kg^{-1} by 1 g kg^{-1} steps (0.1 to 10% by weight). The training set of the Mix model comprised all samples made for treatments LDPE, PET, and PVC both from their training and testing sets.

2.2.3.2 Testing sets

The treatments for LDPE, PET, and PVC each had a testing set of 50 samples plus five of the pure soil controls. The amount of plastic and soil added to the pots, and thus the final concentration of microplastic of each sample, was defined by randomly selecting concentrations within the range of 1 to 100 g kg^{-1} without replacement. The Mix treatment testing set had twenty samples plus five pure soil controls. Randomized amounts of the three plastic polymers (LDPE, PET, and PVC) were added to the aluminium pots for a total microplastic concentration in each sample of less than 80 g kg^{-1} (8.0% by weight). Microplastic weights for this treatment were weighted with a ±0.01mg precision scale (model 210P Sartorius, Göttingen). The total weight of each sample was set to 9 g, adding the needed soil weight to the pots.

2.2.4 Vis-NIR spectral acquisition

General recommendations for soil analysis using vis-NIR Spectroscopy were followed (Wetterlind et al., 2013). Samples vis-NIR spectra were recorded using a portable spectroradiometer with a working range of 350 to 2500 nm (FieldSpec® 3 Analytical Spectral Devices, ASD Inc., CO). The spectroradiometer had a spectral resolution of 3 nm for the 350–1000 nm region and 10 nm for the 1000–2500 nm region, recording the spectrum with a 1 nm interval. A contact probe with a built-in halogen bulb was attached to the device. The probe allowed for direct measurements through a spot size of 10 mm. A Spectralon® white reference panel was used to calibrate the instrument every ten minutes. Dark current measurements were made within the same time interval. For each sample, an average of 100 measurements was recorded as one independent reading.

Before recording the spectra, soil samples were homogeneously mixed with a stainless steel spoon and their surfaces were levelled. Five independent readings were recorded per sample and the probe placed in different points following a quincunx. The five recordings were averaged, obtaining a unique spectrum per sample. This procedure was performed three times for the samples in the testing sets of each treatment, producing three spectral replicates per sample within each testing set (Fig. 2.1). The complete reading procedure was performed three times for the training set of the Mix treatment in order to evaluate the method repeatability (section 2.2.5.3 and 2.2.5.4). The spectra of the pure plastic materials were recorded as reference. All spectra were recorded as vis-NIR reflectance (%). Before the statistical analysis, the spectra were centred and scaled by their variance. Additional pre-treatments such as differentiation or transformation to apparent absorption were not needed.

2.2.5 Data analysis

2.2.5.1 General spectra characterization

General spectral characteristics of the three polymers used were evaluated descriptively. The spectra acquired for the bare soil and the pure plastics were described visually and major trends individuated. The interaction between soil and the different amounts of added plastics was inspected to determine if the bare soil spectra presented changes because of the increasing amounts of added plastics and, if so, to which extent. Evident changes in soil spectra with increasing amounts of added plastic were notated.

2.2.5.2 Predictive model

A Bayesian approach to a multiple linear regression was used to predict the plastic content of a soil sample using its spectral data. This approach has proved to be useful in studies where a large number of predictor variables outnumber the observations. The whole procedure and detailed description of software and available models can be found in Pérez and De Los Campos (2014). Separate models where fitted to each treatment according to their plastic polymer. For the Mix treatment, three models were evaluated independently, one for each type of polymer, setting the added amount of the polymers not being evaluated to 0 (Mix [LDPE], Mix [PET], and Mix [PVC]). Using the spectra acquired for the training samples, the added amount of microplastic y_i was regressed on the standardized spectra using the linear model

$$y_i = \beta_0 + \sum_{j=1}^{2150} x_{ij}\beta_j + \varepsilon_i$$

where β_0 is the intercept (the expected value of y when the wavelengths are set to their means), $\{x_{ij}\}$ is the reflectance at each wavelength for a given sample i, β_j is the effect of the wavelengths, and ε_i is the error term, assumed to be normally distributed with mean zero and variance σ_ε^2. Following this assumptions, the conditional distribution for the added microplastics to the samples $P(y|\theta 0$ is

$$P(y|\theta) = \prod_{i}^{n} N(y_i|\beta_0 + \sum_{j=1}^{2150} x_{ij}\beta_j, \sigma_\varepsilon^2)$$

Where $y = \{y_i\}$ represents the added amount of microplastics of all soil samples given θ, which stands for the collection of all the model parameters (β_0, $\{\beta_j\}$, and σ_ε^2). The relation is given by a normal distribution with mean $\beta_0 + \sum_{j=1}^{2150} x_{ij}\beta_j$, and variance σ_ε^2.

The model parameters (θ) are estimated by a probability function, also known as prior density distribution or prior. The prior allows drawing samples from a posterior density distribution when is used jointly with the conditional distribution (or likelihood). The posterior density distribution corresponds to the estimated plastic contents for a given treatment. Here, the prior density for the model parameters (θ) was as follows

$$p(\theta) = N(\beta_0|0, 1e^{05}) + \chi^{-2}(\sigma_\varepsilon^2|df_\varepsilon, S_\varepsilon) + \prod_{j=1}^{2150} p(\beta_j)\,, where \ldots$$

$$p(\beta_j) = t(\beta_j|5, S_\beta)Ga(S_\beta|rate, shape) \cdot Ber(\pi|p)beta(p|shape_1, shape_2)$$

In this prior, the intercept (β_0) is estimated from a normal distribution (N) of mean 0 and large variance (1e05), treating this intercept as a fixed effect. As the wavelengths were scaled and centred, the intercept represents the predicted value of y when the predictor values $\{x_{ij}\}$ are set to their means. The variance (σ_ε^2) is estimated from a scaled-inversed χ^2 with df_ϵ degrees of freedom and S_ϵ scale parameter. Finally, $\{\beta_j\}$ are estimated from a joint probability of a scaled-t density and a point of mass at zero. By this means, β_j parameters are drawn from a scaled-t density distribution with 5 degrees of freedom, and a scale parameter S_β that is drawn from a gamma (Ga) distribution with rate and shape as parameters. The draws are turned on/off ($\pi = 0 \text{ or } 1$) according to a Bernulli distribution (Ber) with the p parameter drawn from a beta distribution that has itself shape_1 and shape_2 as parameters. The last step induced variable selection. The prior used here is usually referenced as BayesB and has proven to work sufficiently in complex spectra problems (e.g. Ferragina et al., 2015).

The BayesB model is implemented in the R environment (R Core Team, 2020) package 'BGLR' (Bayesian Genomic Linear Regression) by Pérez and De Los Campos (2014). The authors provided a comprehensive list of the algorithms implemented, and a list of working examples. The software provided a series of rules to estimate all the high-order hyperparameters of the model ($df_\epsilon, S_\epsilon, S_\beta, \text{rate}, \text{shape}, \text{shape}_1, \text{shape}_2$) that need to be specified. The rules were stablished to produce uninformative but proper priors (does integrate to one). The software drew estimates from the posterior distribution using a Gibbs sampler with scalar updating, meaning that all β_j estimates were drawn within one step. As the distribution of $\{\beta_j\}$ estimates did not have a closed form, their samples were drawn using a Metropolis-Hastings algorithm. The Metropolis-Hastings algorithm implemented by the BGLR package is described step by step in Meuwissen et al. (2001).

Inferences (estimates of $\{y_i\}$) were based on 1e06 samples collected form the posterior after discarding 1e05 samples. The convergence of the posterior chains to a stationary state

was evaluated following the Gelmans and Rubin's approach using three chains. The method is implemented and fully described in the R package 'CODA' (Output Analysis and Diagnostics for MCMC) (Plummer et al., 2006).

2.2.5.3 Statistical analysis

The samples comprised in the testing set of each treatment were measured three times to predict three probable added amounts of plastic per sample using the model (section 2.2.4 and 2.2.5.2). As a Bayesian regression was used, the expected values of $\{y_i\}$ were not unique, but a normal distribution with mean $\{\hat{y}_i\}$ and standard deviation $\{\sigma_{yi}\}$ (section 2.2.5.1). A Monte Carlo approach was used to average the three inferences out from the three spectral repetitions, drawing 1000 scenarios out of each of the three expected values of $\{y_i\}$. The simulated predictions were averaged and the interquartile range (IQR) calculated to report a final result (Fig. 2.1). The testing set of the Mix treatment were counted with three final results per each sample, as the whole reading-predicting procedure was performed three times per sample.

2.2.5.4 Assessment of prediction accuracy

The accuracy in the prediction of each treatment was addressed using the testing sets. The linear relationship between the added plastic as concentration and those predicted by the model ($\{\hat{y}_i\}$) was evaluated. Firstly, a linear regression was adjusted to the added and predicted concentrations, calculating the slope of the linear relationship (m) and the coefficient of determination (R^2). Secondly, the Pearson correlation between the added and predicted concentration was observed. Finally, the root-mean-square deviation (RMSD) was calculated as $RMSD = \sqrt{\sum(\hat{y}_i - y_i)^2/N}$, where y_i is the added plastic as concentration and $\hat{y}_i$ the predicted by the model for a sample i, with N number of samples. The RMSD stands here as a descriptive measure of the differences between the model predicted values and the added concentrations of plastic. The RMSD approach is widely suggested for vis-NIR model assessments (Wetterlind et al., 2013). In addition to the linear relationship between the variables, the model residual variance (σ_ϵ^2) and the expected value of y_i standard deviation ($E[\sigma_y]$) were used to compare between the models from the different treatments.

The method detection limit was defined as the concentration of plastic in g kg^{-1}, which gives a predicted concentration equal to three times the standard deviation of the predicted concentration of the bare soil samples (blanks) included in the testing set of treatments.

The testing set of the Mix treatment was used to evaluate the repeatability of the method, since three final results were obtained for each sample. The coefficient of variation (CV) was calculated (standard deviation / mean) for each final prediction of added microplastic,

using the results from all of the 20 samples to estimate the descriptive statistics of the CV (quantiles, mean, standard deviation). Furthermore, the coefficient of repeatability (CR) was assessed as the 95% confidence interval for expected differences in the final prediction of the same sample.

2.3 Results

2.3.1 Spectral characteristics of the different plastics polymers and their interaction with soil

The spectra recorded as reference for the plastic polymers revealed that the three plastics presented different behaviours along the studied wavelength range (Fig. 2.2). Furthermore, the bare soil spectral signature stood out without overlapping with any of the plastics studied. The only exception was within the 650 to 800 nm range, in which the soil signal joined that of PET. While all three plastic polymers presented a lower reflectance than the soil at the shortwave infrared range (from 1700 nm onwards), the behaviour at the near infrared and visible range varied among polymers.

Low-density polyethylene presented a high reflectance within the visible range diminishing while the wavelength increased. It was the brighter of the three plastic polymers evaluated and was brighter than the soil within the visible and near infrared range. It presented three major and distinctive absorption peaks at 1210, 1420 and 1730 nm, and two minor peaks at 930 and 1040 nm. Polyvinyl chloride presented the higher absorption among all plastic polymers studied. Its reflectance was consistently lower than that of the soil, decreasing steadily through the wavelength range. Reflectance values for PVC were below 13% across the whole range studied, shrinking below 10% at wavelengths above 1000 nm. This plastic presented two subtle absorption peaks at 1720 and 2300 nm. Finally, PET fell between PE and PVC. Within the visible range, PET presented a higher reflectance than that of the soil, overlapping the soil signal between the 650 and 800 nm when it reached its higher reflectance (43%). At wavelengths greater than 800 nm, PET reflectance started to decrease progressively until it merged with LDPE and PVC signals at 2300 nm (reflectance = 8%). Polyethylene terephthalate presented distinctive absorption peaks at 1210, 1420 and 1730 nm matching LDPE behaviour.

Soil samples with added microplastics presented changes respective to the bare soil spectral signature along all of the wavelengths recorded (Fig. 2.3). While there were virtually no distinctions within the visible range up to 800 nm, at higher wavelengths, the soil reflectance contracted proportionally to the amount of plastic added as pollutant. In this way, the soil reflected less light with increasing plastic concentrations at the near and

shortwave infrared range. As the data shows, the proportion of the reduction in the reflectance due to the plastic addition depended on the wavelength at which the reflectance was measured and the plastic polymer added. On the one hand, since all plastics decreased their reflectance when the wavelength increased, the proportion of the reduction was higher when the measurements were taken at longer wavelengths. On the other hand, plastics determined soil reflectance in different degrees as they have distinctive optical properties. For example, at a given wavelength and similar plastic concentration, PVC decreased soil reflectance to a higher degree than LDPE, due to PVC's stronger absorption. Congruently, the stronger absorption peaks observed for LDPE were reflected by the spectral signature of a soil polluted with this polymer.

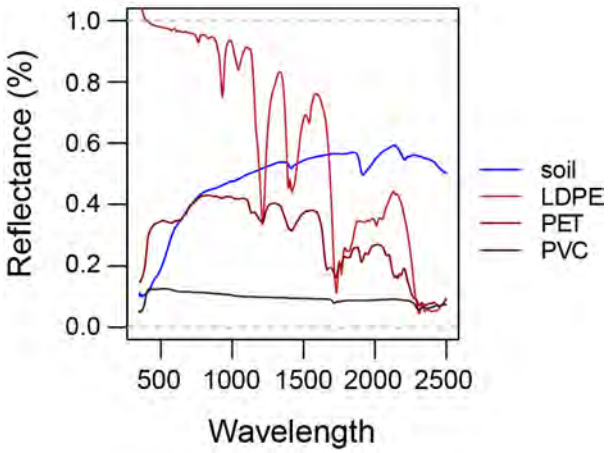

Figure 2.2 *Spectral characteristics of the soil and the different plastics polymers used. Reflectance of pure references (100%) of LDPE, PET, and PVC and soil.*

2.3.2 Predicting added microplastic concentrations in soil samples

The model performance presented the highest correlation coefficient and the best fit for the LDPE treatment (Table 2.2). This treatment also showed the lowest residual variance (σ_ϵ^2) and expected value of y_i standard deviation ($E[\sigma_y]$) among the treatments that comprised one plastic polymer and had an equally-sized training set (LDPE, PET, and PVC). The slope of the linear relation (m) between the added plastic and the predicted concentration suggested a slight underestimation of the soil plastic content. Fig. 2.4 indicates that the underestimation of LDPE increased as the amount of plastic added increased. The RMSD shows that predicted concentrations had a standard deviation from the added amount of 8 g kg^{-1}, which represents around the 8% of the concentration range studied (0 – 100 g kg^{-1}). A direct implication is that predictions of LDPE concentrations below

10 g kg^{-1} could lead to false positives. Nevertheless, the detection limit estimated for LDPE was 3 g kg^{-1}.

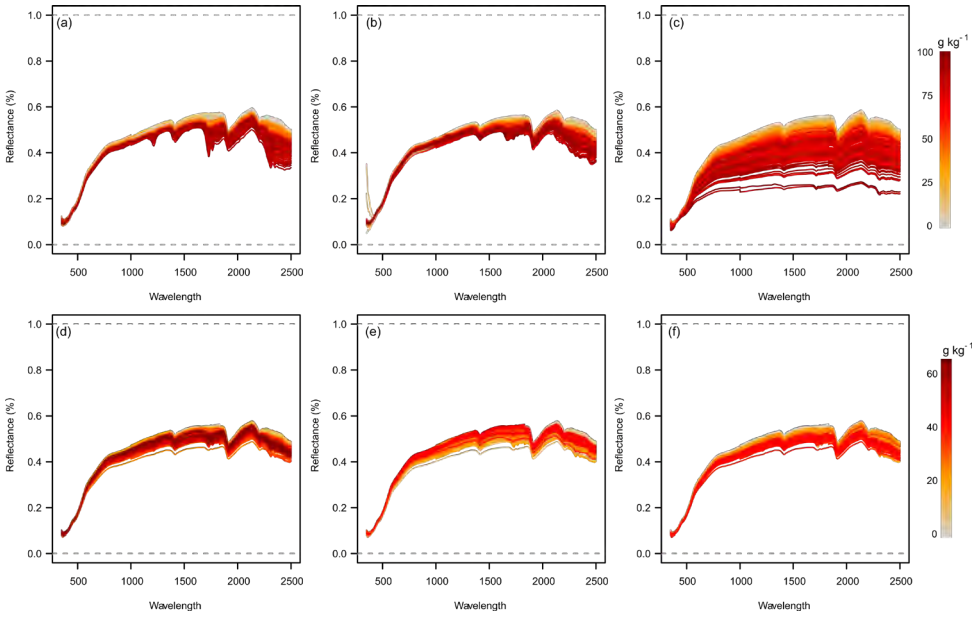

Figure 2.3 *Changes in the Vis-NIR spectrum of a soil when increasing amounts of (a) LDPE, (b) PET, or (c) PVC are added, and when a combined amount of the same polymers are spiked (d-e), tracing (d) LDPE; (e) PET; (f) PVC one at a time (Mix treatment).*

The model performance for PET showed the lowest fit across all treatments. Yet, the correlation was significant and close to 0.80 and although the σ_ϵ^2 was higher than for LDPE, it stayed close to that of PVC. Contrariwise, the $\mathrm{E}[\sigma_y]$ was large, standing out with a figure that approached 10% of the concentration range studied. The observed $\mathrm{E}[\sigma_y]$ reflects back to a large RMSD, which is detached from all other treatments with a value twice as large. Fig. 2.4 suggests that the goodness of fit decreased at PET concentrations above 60 g kg^{-1} and that there were outliers within the samples. On one hand, as the samples were custom-made by adding a precise amount of plastic, the outliers were kept since they reflect PET properties and not laboratory mistakes. On the other, the model performance was assessed a second time pruning the predictions above 60 g kg^{-1}. Evaluating the predictions within the pruned concentration range increased the model fit (R^2 = 0.89) and the correlation (Pearson's r = 0.95), and decreased the RMSD (7 g kg^{-1}) considerably.

PVC treatment stood close to LDPE, showing a similar fit and correlation. Nonetheless, the σ_ϵ^2 was the highest of all treatments. The observed $\mathrm{E}[\sigma_y]$ was larger by about 2 g kg^{-1}

compared to that of LDPE and 4 g kg^{-1} smaller than that of PET. The RMSD shows that predictions had a standard deviation from the added PVC around 2 g kg^{-1} larger than that of LDPE. Once again, the RMSD suggests that predictions of PVC below 10 g kg^{-1} could lead to false positives. In the same direction, Fig. 2.4 indicates that predictions of added values below 25 g kg^{-1} adjust poorly to a 1:1 linear relation. Similarly to LDPE, PVC treatment did not show extreme outliers.

Table 2.2 *Indicators of prediction accuracy for each treatment. Coefficient of determination (R^2), linear relation (m), root-mean-square deviation (RMSD), Pearson's r (Correlation), model residual variance (σ_ϵ^2), expected value of y_i standard deviation (E[σy]), and detection limit (DL).*

Treatment	R2	m	RMSD	Correlation	σε2	$E[\sigma_y]$	DL
			g kg-1		---------- g kg-1 ---------		
LDPE	0.96	1.13	8	0.98	0.064	5	3
PET	0.62	1.24	18	0.79	0.148	11	26
PVC	0.90	1.13	10	0.95	0.154	6	21
Mix [LDPE]	0.79	1.25	8	0.89	0.057	2	15
Mix [PET]	0.82	1.66	10	0.90	0.071	1	12
Mix [PVC]	0.89	1.28	5	0.94	0.057	2	14

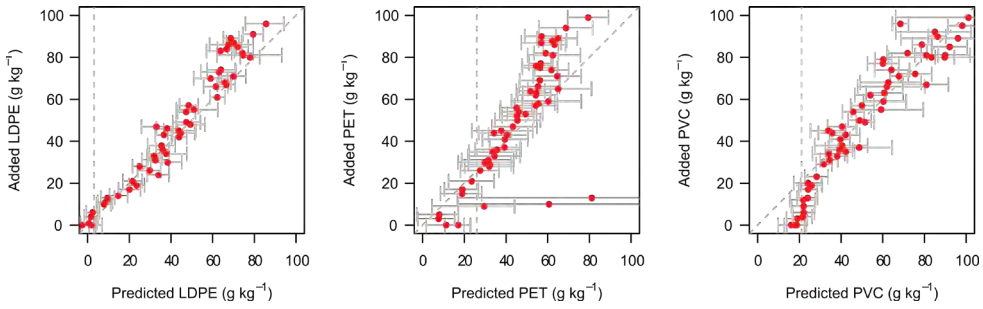

Figure 2.4 *Predicted microplastic concentrations in samples against added microplastic weights for LDPE (left), PET (centre) and PVC (right) treatments. The red dots stand for the predicted mean, while the grey bars stand for the ±IQR. The diagonal dashed grey line corresponds to the 1:1 linear relation, while the vertical corresponds to the detection limit.*

2.3.3 Predicting the concentration of a single microplastic polymer in soil samples with more than one polymer type

In general terms, the Mix treatment had a lower goodness of fit than LDPE, PVC, and the pruned PET (Table 2.2). Within this treatment, the correlation between the added amounts of microplastics and the predicted value fluctuated around 0.90, which was not far from the Pearson's r observed for LDPE and PVC. Moreover, the σ_{ϵ}^2 was on average three times smaller than that of PET and PVC due to the larger number of samples included in the Mix training dataset respect to the remaining treatments. This is equally applicable to the $\mathrm{E}[\sigma_{\mathrm{y}}]$, which was consistently smaller for all plastic polymers when they were predicted under the Mix scenario (more than one plastic polymer present in the sample) respect to the remaining treatments.

Low-density polyethylene presented the lowest fit when its concentration was predicted under the extra noise of the Mix treatment (Table 2.2, Mix [LDPE]). Nonetheless, the Mix [LDPE] predictions presented a significant correlation with the added plastic, which was similar but slightly lower than that of Mix [PET] and Mix [PVC]. The σ_{ϵ}^2 was the lowest of all treatments, matching that of Mix [PVC]. The $\mathrm{E}[\sigma_{\mathrm{y}}]$ was considerably lower than that observed when low-density polyethylene was predicted in the LDPE treatment because of the larger training set. Its value was, however, the highest among the plastic types predicted within the Mix treatment. The observed RMSD was equal to that of LDPE, and was 2 g kg^{-1} smaller than that of Mix [PET]. Regarding the repeatability, Mix [LDPE] predictions had the lowest coefficient of variation (CV) of the Mix treatment, which presented a 3% interquartile range (IQR) with a median of 8% (Table 2.3). The coefficient of repeatability (CR) showed that predictions of low-density polyethylene concentrations in soil samples polluted with more than one polymer were expected to vary up to 8.4 g kg^{-1} for the same sample. Fig. 2.5 shows that the Mix [LDPE] predictions did not deviate from the 1:1 ratio with the added low-density polyethylene, and that there were no outliers within the treatment.

Mix [PET] predictions showed a higher correlation with the added concentration of plastic and a better fit compared to the PET treatment (Table 2.2, Mix [PET]). The best goodness of fit echoed in a relatively lower RMSD for Mix [PET] than that of PET. The CV presented an interquartile range of 18% with a median of 20% (Table 2.3). This was the highest CV observed for all plastic polymers predicted for the Mix treatment. However, the CR for Mix [PET] was the lowest observed, indicating that the expected variation of repeated measurements will most probably be under 5.1 g kg^{-1}. Despite the higher accuracy in repeatability that Mix [PET] presented compared to Mix [LDPE] and Mix [PVC], its predictions continuously underestimated the amount of plastic added to the soil sample,

as was revealed by the linear 1:1 relation (Fig. 2.5). In this regard, the observed value of m was the larger among treatments (Table 2.2).

Table 2.3 *Repeatability indicators for Mix treatment. Minimum, maximum, quantiles, and mean for the observed coefficient of variation for the same sample. Coefficient of repeatability (CR) in g kg^{-1} as the 95% confidence interval for differences in predictions for the same sample.*

Treatment	Coefficient of variation (%)						CR		
	Min	1st Qu	Median	Mean	3rd Qu	Max.	SD	p = 0.025	p = 0.975
Mix [LDPE]	1	6	8	8	9	23	5	0.2	8.4
Mix [PET]	3	12	20	25	30	90	22	0.1	5.1
Mix [PVC]	3	9	15	15	20	33	8	0.1	9.0

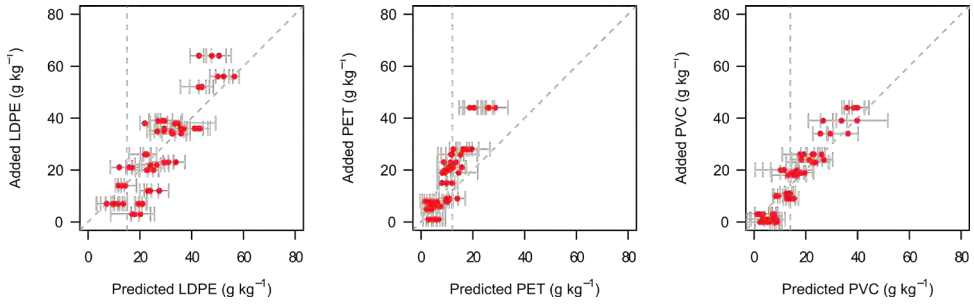

Figure 2.5 *Predicted LDPE (left), PET (centre), and PVC (right) concentrations in samples against added weights for the Mix treatment. The red dots stand for the predicted mean, while the grey bars stand for the ±IQR. The diagonal dashed grey line corresponds to the 1:1 linear relation, while the vertical corresponds to the detection limit.*

Mix [PVC] predictions presented the best fit and the highest correlation with the added microplastic among all Mix treatment plastic assessments (Table 2.2, Mix [PVC]). The RMSD observed was the lowest among all treatments. However, the repeatability was weaker than that of Mix [LDPE] showing a CV with an interquartile range of 11% and a median of 15%. The CR was the broadest observed for Mix predictions, indicating that repeated measurements are expected to vary up to 9.0 g kg^{-1}. Fig. 2.5 shows a good adjustment between the predicted concentrations and the added amount plastic to the 1:1 linear

relation, although the observed m value indicates a tendency to underestimate the added plastic content.

2.4 Discussion

2.4.1 vis-NIR qualitative and quantitative prediction capabilities

The vis-NIR method proposed was able to predict microplastic concentrations of LDPE, PET, and PVC in soil samples, presenting an approximate accuracy of 8, 5, and 9 g kg^{-1} (upper boundary of the repeatability coefficient) and a detection limit of 15, 12, 14 g kg^{-1} for LDPE, PET, and PVC, respectively. The method was fast, taking about three minutes to complete each independent reading (five spectra acquisition). Moreover, it was able to predict the microplastic concentration in samples that had composite amounts of microplastics, showing qualitative and quantitative analytical capabilities. This was different than visual identification techniques and FT-IR and chromatographic techniques such as Pyr-GC-MS or the more recently proposed thermal desorption gas chromatography mass spectrometry (TED-GC-MS) (Dümichen et al., 2017b).

On the one hand, visual identification techniques attempt to count or estimate the number of microplastics over a given area, resulting in a number of particles detected in a standard volume of sample (Crawford and Quinn, 2017b). This leads to possible biases due to the presence of misleading organic or clay particles that might be wrongly counted as plastics (de Souza Machado et al., 2018a; Ziajahromi et al., 2017). While this drawback has been partially overcome by semi-automated computer estimations, there is still a point at which a human operator needs to decide what on the image to be processed is plastic and what is not (Zhang et al., 2018). Moreover, visual techniques cannot qualify microplastics by compound and can only classify different particles by colour, size, and shape (Hidalgo-Ruz et al., 2012). Therefore, visual techniques usually rely on additional qualification steps performed by FT-IR (Mahon et al., 2017).

On the other hand, FT-IR deals with microplastics isolated from bulk samples. This method is useful to qualify the plastic type that is present within a sample. Nonetheless, FT-IR instruments were slow in acquiring the spectral images, even when equipped with a focal plane array that increases the sample area to be measured (Mintenig et al., 2017). Moreover, samples containing multiple microplastics polymers are known to constitute a challenge for FT-IR spectroscopic analysis (Fuller and Gautam, 2016), but new automated approaches offer a solution to the problem of identification (Primpke et al., 2017). In this regard, efforts have been recently made to not only qualify but to quantify the microplastic content (Simon et al., 2018). The opportunity that vis-NIR spectroscopy offers, however, is

that it allows the quantification and qualification of the microplastic content in soil samples using one analytical step.

However, the use of vis-NIR spectroscopy is not free from the challenge of polymer recognition. The predictions made by the spectroradiometer method worked better when homogeneously coloured plastics were added to the samples (LDPE, PVC). Prediction accuracy was diminished when multi-coloured plastic was used (PET). Similarly, interference due to dyeing molecules has been reported in other techniques of microplastic recognition (Lots et al., 2017). Besides the higher RMSD observed for PET, the drop in prediction accuracy was also revealed by the increase in the deviation of PET predictions (E[σy]). The decrease in accuracy is an obvious limitation because a bigger spectral library (training set) that can take different colours/dyes into account is needed to avoid colour-related-noise, as will be discussed in section 2.4.3.

The use of NIR spectroscopy has recently been tested by (Paul et al., 2019). In this work, the authors used an spectrometer to predict whether a soil sample held PE, PET, polypropylene, polystyrene, and/or PVC. Despite they have used a different statistical approach, a similar dependency on the training set was found. Thus, made-to-measure reference samples are needed to avoid false positives (see section 2.4.3). Besides the similarities, the method described by Paul et al. (2019) was not able to predict plastic content, and was limited to classifying the samples as positive or negative for the presence of microplastics. Therefore, the quantitative prediction capabilities showed by the regression approach constitute a step forward for microplastic detection in soil samples.

2.4.2 Avoidance of microplastic extraction in vis-NIR techniques

The use of the spectroradiometer proposed in this work circumvents the need for microplastic extraction. Moreover, while only minimal sample preparation (drying and sieving) was carried out during this study, spectroradiometers could be used to measure bulk soil samples thus avoiding extensive sample preparation (Xu et al., 2018). The avoidance of microplastic extraction steps gives rise to two major differences with respect to current techniques. Firstly, it reduces the total time devoted to sample analysis, making the overall analytical time shorter. Secondly, it removes the need for sample preparation and this reduction in manipulation diminishes biases caused by human handling (Ziajahromi et al., 2017).

The extra steps commonly required for sample preparation increase the analytical time. Flotation methods take between 12 and 24 hours to complete not to mention the time needed to dry samples, filters, and sieves (Lots et al., 2017; Zhang et al., 2018). Pressurised fluid extraction remains a fast alternative (15 min), but this method is not perfect and can

result in recovery rates greater than 100% (Fuller and Gautam, 2016). To date, elutriation has not been tested on soil samples, but the total processing time to isolate microplastics from sand particles takes up to 30 minutes (Claessens et al., 2013). Furthermore, when the elutriation extraction was tested on samples rich in organic matter (sludge), the overall processing time was longer (Mahon et al., 2017). The complexity of wet separation methods has pushed researchers to explore new techniques. The use of electrostatic separators is one of the most recent techniques (Felsing et al., 2018). Despite the fact that it has a promising future, the extraction itself takes 4 hours to complete and it has not been tried in soil. Therefore, skipping the extraction steps and reducing sample preparation time are definitely advantages to using spectroradiometers.

However, current extraction techniques could lead to biases caused by sample manipulation. Flotation, fluid extraction, and elutriation involve many steps consisting of watering, sieving, and filtering as well as the intermediate steps of brushing particles from filters or sieves onto microscope slides, petri dishes, or centrifuge tubes (Fuller and Gautam, 2016; Mintenig et al., 2017; Zhang et al., 2018). Reported recovery rates indicate around 10% from interferences. Authors frequently claim that the sample preparation steps are not standardized which makes dealing with complex matrices such as soil challenging to researchers. Here again, the use of spectroradiometers to directly measure microplastic content in soil samples is an advantage.

2.4.3 Method limitations

The need for a training set to predict the content and type of polymers within a soil sample constitutes a limitation for the proposed method. A comprehensive training data set is necessary in order to expand the technique further to encompass other polymers and concentration ranges. Moreover, as vis-NIR spectra varies for different soil types, increasing or decreasing the amount of reflected light, different soil types should also be considered when an analysis is performed (Viscarra Rossel et al., 2016). Therefore, a made-to-measure training library is needed to establish a calibration curve for each scenario to be predicted. The method success rates rely on the meticulous construction of this calibration curve. Thus, exploratory analyses are needed to understand which plastic polymers are to be expected and at which concentration ranges.

Despite the fact that generating a made-to-measure training dataset for a given scenario could be time consuming, the advantage is that once constructed, it could be used indefinitely. This is similar to the spectral libraries that use the FT-IR instruments to identify different polymers (Crawford and Quinn, 2017b). Furthermore, acquired sets could be enhanced by new additions since the results showed that larger the training data, the better the prediction.

Another limitation could be the crude predictions of microplastic concentrations (RMSD ≈ 10 g kg^{-1}) and detection limits ≈ 15 g kg^{-1}. Worldwide, there is a lack of environmental monitoring campaigns reporting expected concentrations of microplastics in soils (Bläsing and Amelung, 2018), making it difficult to know if the assessment capability of the method proposed could be useful for general monitoring purposes. Table 2.4 presents some of the values reported so far.

According to the information reviewed, the proposed method would only be suitable for the study case used in the work of Fuller and Gautam (2016). However, theoretically, it would be also applicable under some of the critical scenarios projected by Ng et al. (2018). All of the cases examined microplastic hotspots. While Fuller and Gautam (2016) evaluated soil samples located near an industrial area, Ng et al. (2018) estimated expected concentrations of plastics for soils with high rates of sludge application. Although the pathways by which microplastics reach the soil are yet unclear (Vollertsen and Hansen, 2017), there is an increasing assumption that sludge application could be one of the leading routes (Nizzetto et al., 2016b). Therefore, the method could be used to rapidly quantify and qualify microplastic content in hotspots such as industrial and dump sites and arguably in agricultural soils with recurrent sludge applications. Consequently, the proposed vis-NIR technique could be coupled to monitoring campaigns where other more precise but time-consuming strategies are used.

Noticeably, the use of the proposed method in hotspots should be done with care. Vollertsen and Hansen (2017) studied microplastic occurrences in Danish soils either with or without sludge application and found only trace concentrations of microplastics in both management scenarios. Thus, it is not always true that sludge applications naturally imply the presence of a microplastic hotspot. Furthermore, in China's Loess plateau, where plastic mulch has been used for almost 20 years to cultivate crops, the concentration of microplastics in soil samples measured a maximum of 0.1% (Zhang et al., 2018).

While the avoidance of extraction procedures constitutes and advantage of vis-NIR spectroscopy, the detection limit could be lowered using concentration steps. While carrying out the separation steps increases the total analytical time, the inherent reading speed of the spectroradiometer results in faster analysis than performing particle image evaluations. New developments in dry extraction using electrostatic separators are promising (Felsing et al., 2018) and should be tested in soil. To this propose, new separator prototypes must be designed, taking into consideration both the sample volume and the processing time.

We propose that by coupling the presented methodology with a proper separation step, the method could be used not only in hotspots, but for general microplastic quantification

and identification in soil samples. As an example, we conducted a short trial to test how far the quantification capabilities can go if the polymers are isolated from the matrix (soil, sludge, etc) before measuring with the spectroradiometer. Using activated charcoal filter paper (Macherey-Nagel MN 728) as a background media, the reflectance of increasing amounts of each polymer was recorded. Following the same statistical approach, the method was able to predict in milligrams the amounts of microplastics particles deposited over the filter. The test showed a DL of 0.7, 1.2, and 1.0 mg for LDPE, PET, and PVC, with a RMSD of 0.7, 0.7, and 1.1 mg, respectively (Fig. 2.6). The accumulation of the plastic particles over the filter could be achieved by flotation and filtration steps at the end of which the particles remain trapped within the filter. Nevertheless, the coupling of the method with separation steps needs to be studied further, as the matter exceeds the expectations of the present work.

Table 2.4 *Reported concentrations of microplastics in soil samples. Lower (L) and higher (H) concentrations reported in %. The methods used for extraction and quantification are included (Extraction & Quantification).*

Author	L	H	Place	Extraction & Quantification
Fuller and Gautam (2016)	**0.03**	**6.75**	**Australia**	**Pressurised fluid extraction & FT-IR**
Zhang et al. (2018)	**<0.01**	**0.11**	**China**	**Flotation & Semi-automated visual identification**
Huerta Lwanga et al. (2017b)[a]	**-**	**-**	**Mexico**	**Flotation & Visual identification**
Vollertsen and Hansen (2017)	**<0.01**	**<0.01**	**Denmark**	**Flotation FT-IR**
	<0.01	**<0.01**	**Denmark**	**Flotation FT-IR**
Ng et al. (2018)[b]	**1.44**	**9.88**	**Australia**	**Theoretical estimation**
	0.31	**2.00**	**USA**	**Theoretical estimation**
	0.03	**0.39**	**Europe**	**Theoretical estimation**

[a] Authors reported microplastic concentrations in number of particles per gram (0 - 2.77 particles g-1). Therefore, there is not a direct way to estimate the concentration.

[b] Ng et al. (2018) reported theoretical input values of microplastics to soils through sludge application. The maximum value reported corresponds to an extrapolation of the highest rate disposed after 15 years of sludge application.

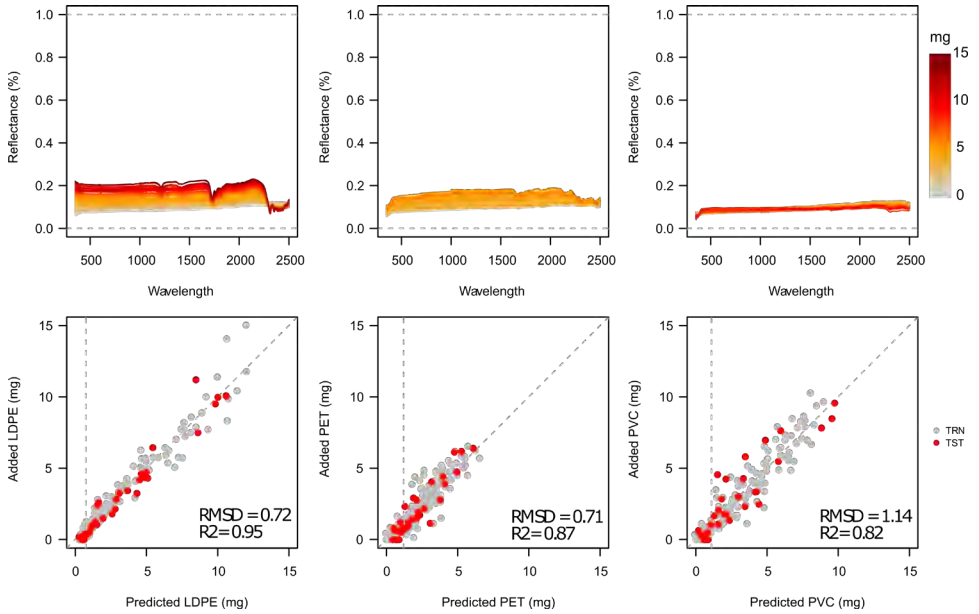

Figure 2.6 *Top: change in the reflectance of activated charcoal filter paper when increasing amounts of LDPE (a), PET (b), and PVC (c) are added to the filter. Bottom: predicted weights of LDPE (d), PET (e), and PVC (f) added to the filter against the known weights. A hundred and fifty samples (i.e. filters + a known concentration of polymer) were register per polymer, using 120 records as a training group (grey dots) and 30 records as a testing set (red dots). Validation was performed using the testing set. Dotted grey vertical lines indicate the DL for each polymer, while diagonal stands for the 1:1 linear relationship.*

2.5 Conclusion

Our results suggest that it is possible to use vis-NIR techniques to identify and quantify LDPE, PET, and PVC microplastics in soil samples. To this purpose, a spectroradiometer with a working range from 350 to 2500 nm can be used to predict microplastic concentrations with an accuracy of 10 g kg^{-1} and detection limit of ≈ 15 g kg^{-1}. The method proposed is different than other approaches, being faster, avoiding extraction steps, and directly quantifying the amount of microplastics in a sample.

Our work constitutes a proof of concept in using vis-NIR techniques to qualify and quantify microplastics in soil samples. However, there is a lot to uncover in order to develop a useful technique that can be applied to multiple scenarios. The development of a general purpose spectral library will be one of the most challenging milestones in developing this method. On the bright side, a comprehensive spectral library could be built progressively and include smaller libraries compiled for more specific goals. Direct on-site measurements of microplastics in soil at hotspots (>15 g kg^{-1}) should be tested, exploring the possibility of the complete suppression of sample preparation. Finally, as a proof of concept, this work provides an opportunity for other applications or devises that use vis-NIR spectra acquisition -such as hyperspectral cameras- to be used in studies that deal with microplastics.

Chapter 3. uFTIR: an R package to process hyperspectral images of environmental samples captured with µFTIR microscopes

uFTIR is an R package that implements an automatic approach to analyse µFTIR hyperspectral images with a strong focus on microplastic recognition in environmental samples. The package performs image classification using a Spectral Angle Mapper algorithm in a library search approach. It interacts with other R packages used for spectral analysis. It exports its output as raster and vector files that can be post-processed in common Geographical Information Systems software. The package was designed around the principles of modular development, compatibility, and open-source software. We hope our contribution will serve researchers to size the occurrence of microplastics in ecosystems.

Based on:

Corradini, F, N Berriot, E Huerta-Lwanga, V Geissen. 2020. uFTIR: an R package to process hyperspectral images of environmental samples captured with µFTIR microscopes. SoftwareX [Submitted]

Software available at: https://CRAN.R-project.org/package=uFTIR

3.1 Motivation and significance

In the last decade, scientific concerns about environmental pollution by microplastic have scaled up, reached public opinion, and positioned within the political agenda (Foteinis, 2020; Henderson and Green, 2020). With all the evidence that scientists have gathered, it is conceivable that policy-makers will promote routine environmental monitoring programs (Fossi et al., 2020). However, a problem might hamper the development of such initiatives. The same problem that has hindered research for years. Scientists have not agreed on standard methods to quantify or identify microplastics in environmental samples (Alexy et al., 2020; Van Raamsdonk et al., 2020). The problem hampers not only future monitoring initiatives, but it precludes study comparisons and metadata analyses (Li et al., 2020a).

To date, scientists have mainly used single purpose methods and low laboratory automation to address microplastic pollution. The lack of standardized methods rises as a consequence of such approach (Primpke et al., 2017). Available analytical methods propose three step analysis that consider extraction, instrument detection, and particle identification and count. Although standards lack for each of the steps, scientists struggle the most to achieve both particle quantification and particle count using a single method or instrument (Wang et al., 2020a). Commonly, methods focus in one or the other. Visual identification and sorting methods exemplify the problem notably. Scientists use these methods commonly to quantify plastic particles in environmental samples (Li et al., 2020a). Visual identification relies on the trained eye of technicians who tag and sort 'suspected' particles by colour, shape, and other physical attributes (Horton et al., 2017). Since the method does not identify the polymer type, researchers must implement additional analytical steps for polymer identification (Hurley et al., 2018; Zhang et al., 2018). Consequently, laboratory methods become tedious and time-consuming (Jany et al., 2020). To tackle the problem, scientists have proposed workflows that include laboratory automation.

Laboratory automation has two sides; hardware and software. The industry has tackled hardware requirements and scientists have at disposal equipment capable of identifying plastic polymers (Dümichen et al., 2017a; Primpke et al., 2017). Literature reviews that summarize monitoring efforts identify FTIR spectroscopy as the most common method used to identify plastic polymers (Huang et al., 2020b; Möller et al., 2020; Wong et al., 2020). To provide a complete solution, manufacturers couple FTIR spectrometers with microscopes. The use of µFTIR instruments -as they are called- avoids unnecessary steps in sample handling. However, manufacturers do not provide tools to automate the analysis of the output image.

On the software side, companies do not provide built-in solutions to process the output images automatically. Equipment such as Agilent Cary 620 FTIR spectrometer come with a (proprietary) software that has only basic pixel classification features (Agilent Technologies, Resolution Pro Software). Researchers have taken the lead, proposing different approaches to fulfil software requirements. One of the first propositions came from earth sciences and Geographical Information Systems. Harris (2006) proposed a twostep library search that first runs a feature recognition algorithm and calculates spectral end-members and then runs a library search algorithm (Harris, 2006). The method cleans the spectra but loses information when calculating the end-members, thus most of the time bulk searches using *ad hoc* algorithms are —at least— equally successful (Dennison et al., 2004). Modern approaches build on this second alternative; bulk library search (Liu et al., 2019b; Primpke et al., 2017). Library search presents the advantage that it can be adapted quickly through the implementation of new or extended reference libraries, but it can be computationally intensive (Primpke et al., 2018). Recently, researchers optimized the method performance by clustering the spectra before the search, revisiting the end-members idea (Wander et al., 2020). The method, however, has yet to be implemented.

Scientists have implemented a few alternatives to overcome the absence of software officially supported by µFTIR instrument providers. The Systematic Identification of **M**icro**PL**astics in the Environment (siMPle) software summarizes all alternatives to date in one package suit (Primpke et al., 2019a). The software has some limitations. First, it has shortcomings when dealing with large files —a single sample file size starts from 12Gb. Second, the developers restrict the access to the source code, compromising scientific reproducibility and trustworthy analytical methods (Chambers, 2008). Third, the code's obscurantism veils the analytical workflow and forces the user to choose between a finite set of pre-processing steps. The software limits the analytical possibilities to two algorithms to pre-process the data (calculate spectra's first or second derivative) and one algorithm to perform the library search (correlation). These options fall short when compared with typical spectral analysis steps (Raczkowska et al., 2019).

Given the software limitations, we set out to develop a program able to automate the analysis of µFTIR images built on trustworthy and reproducible research principles. Our main goal was to implement a set of front-end tools to analyse the output of µFTIR spectrometers. Our main focus was its application in environmental research, especially for microplastics analysis. We addressed our goal by writing an R package that structures a library search workflow around the principles of modular development, compatibility, and open-source software. In this article we introduce the uFTIR R package architecture, describe its functionality, present a step by step processing of a soil sample, and contrast the results with alternative software (siMPle).

3.2 Conceptualization and requirements

3.2.1 Analytical steps

The analysis of hyperspectral images comprises five sequential steps that we tackled independently. First, the program reads the hyperspectral image, typically depicted as a 3d array, into the memory. Second, the user defines a number of pre-processing steps to reduce signal noise and to avoid bias in subsequent steps. Third, the program runs a matching algorithm that contrasts the hyperspectral image data with a reference library. Fourth, the user defines post-processing needs and executes them. Fifth, the user checks the success of the matching algorithm and summarizes the output. The result presents information on the size and number of particles for each polymer included in the reference library. The uFTIR package allows the user to run or automate each one of the steps. The current version (v0.1.1) of the uFTIR package implements all the analytical steps to process Agilent Resolutions Pro Software (Agilent Technologies, Inc.) outputs files. The Agilent Resolutions Pro Software comes with the Agilent µFTIR Microscope and Bench (Agilent Technologies, Inc., USA) and together constitute Agilent's suit for FTIR microscope analysis.

Agilent's suit for FTIR microscope analysis allows a spectra recording between 3600 and 700cm^{-1} with a collection resolution between 0.5 and 16cm^{-1}. The manufacturer offers three objectives to equip the microscope; 4x, 15x, and 25x, which yield images with a pixel size of 20.6, 5.5, and 1.1 µm respectively. Agilent Resolutions Pro Software can do only pixel wise library search to compare its output with a known reference. The software comes with a (privative) spectral library for plastic polymers identification called ‘poly_8’. Agilent's software stores the images in a file format with special characteristics that we use as input to start the microplastic recognition analysis in uFTIR. In section 3.2.2 we describe the image characteristics.

3.2.2 Input files

In this subsection we present the challenges of extending R reading functionalities to load Agilent's FTIR microscope images. Agilent's FTIR microscope had two main output file formats. In its most simple usage, the microscope takes the spectra of a ‘single tile’; a single hyperspectral image taken at a fixed position. ‘Mosaics’ extends the single tile format to multiple images. Mosaics constitute the working horse of all automation efforts. They allow the user to take hyperspectral images of an area larger than the microscope field of view. When the mosaic approach is used, the user defines *a priori* an area to record. Then, the microscope takes the images and moves its tray until it covers the whole area. As a result,

mosaic images are a record of multiple single images (chunks) with a header that identifies them.

Agilent's output formats pose challenges for post-processing. Agilent's software stores its output in a proprietary file format. It does provide a translation feature to convert the files to ENVI, another proprietary software commonly used to analyse spatial imagery. Currently, the Comprehensive R Archive Network (CRAN) does not register any packages to read Agilent file formats. Although the R package caTools can load ENVI files into memory (Tuszynski, 2020), the problem persists as mosaic files are typically too large to be loaded without processing them first.

3.2.3 Reference library

Library search methods rely on the availability of comprehensive reference libraries. Unfortunately, researchers lack free access to such resources. Primpke et al. (2018) published the first freely available library tailor-made for microplastic identification. The library includes 270 substances manually aggregated in 32 clusters that stand for different plastic polymers. It includes too other polymers commonly found in environmental samples which might cause misclassification, such as chitin, cellulose, and animal fur. Since Primpke et al. (2018) library is the only spectral library freely distributed among scientists, we included it in the uFTIR package as accompanying data.

3.3 Software description

3.3.1 Software Architecture

The scientific context defined in section 3.1 and the characteristics of the input files served as the cornerstone to design the package principles:

- Researchers are the program end-users.
- The program must be modular and accept user modifications.
- The program must be compatible with processing algorithms implemented already for spectral analysis.
- The program must support stepwise checking of module success and user exploration.
- The program must not overload the host memory. Mosaics should be processed in chunks, since they are usually large files for personal computers.
- Memory intensive processes should be parallelized, taking advantage of the chunk-processing approach.

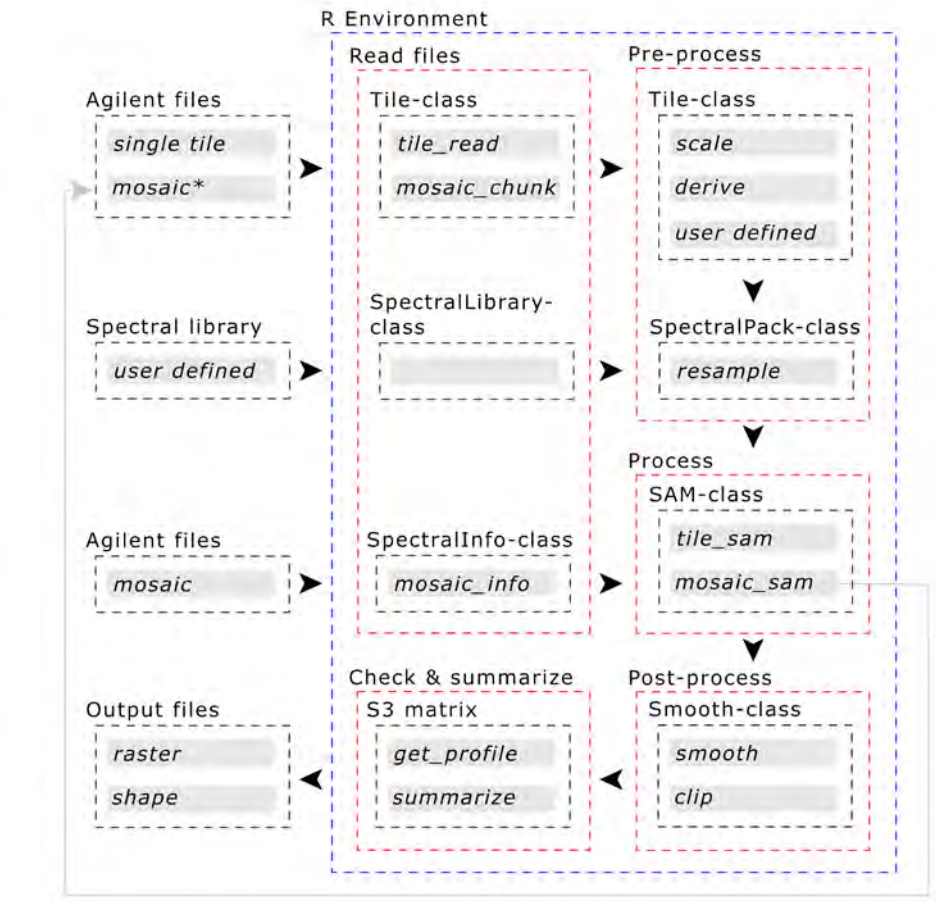

Figure 3.1 *uFTIR R Package architecture. The blue box aggregates the processes that the package performs, all of them inside the R environment. The red boxes individuate each analytical step. The grey arrow shows how mosaic files are read. By calling the mosaic_sam function the user process each mosaic* (sub)file(s) in one call. The summary method returns a three column table with information about the number of particles, their area, and the cluster or substance to which they correspond. The method vectorizes the image that can be saved as ESRI shapefile format.*

In accordance with the design principles, we implemented the application as an R package and defined its output in a format common for geographical information systems (GIS) analysis. This approach has three positive consequences. First, the R environment (R Core Team, 2020; Team, 2019) has a variety of tools implemented already for hyperspectral image analysis. The program can integrate with those, if the user wants to extend the

package built-in features. Second, researchers use the R environment frequently to explore, process, and analyse data. The familiarity that they have with the R environment should soften the learning curve of our software. Third, environmental researchers have at least a common knowledge of GIS and GIS software. GIS allows the user to visualize, manipulate, and process spatial data. Open source libraries and software to work with GIS formats are free and well maintained (see GDAL (GDAL/OGR contributors, 2020), GRASS (GRASS Development Team, 2017), and QGIS (QGIS Development Team, 2009)). Researchers can use these suits to summarize and check the data. The R environment has features to manipulate GIS file formats too.

Figure 3.1 shows the package general workflow. We subdivide the workflow in five sequential steps (see section 3.2.1). We describe each feature and its characteristics in section 3.3.2. Figure 3.3 shows how each step works when processing an environmental sample.

3.3.2 Software functionalities

The R package uFTIR presents the following major functionalities:

Read files. The uFTIR package defines two classes to manipulate Agilent Resolution Pro FTIR files. One reads a single tile directly into memory and the other creates a virtual class that holds the location of all mosaic subfiles. We based the code that implements the reading process on Henderson's MATLAB solution (Henderson, 2017). We translated the MATLAB code to R (single tiles) and C++ (mosaics) to import the reading functionality to R.

The uFTIR package conceptualizes hyperspectral images as 3d arrays. The first two dimensions of the array define coordinates on the microscope tray (commonly called pixels). Each pixel holds, along the arrays' third dimension, the spectrum of the area that matches its coordinates on the microscope tray.

Pre-process. The program implements three methods to pre-process the spectra: scale, calculate first and second derivatives, and resample. The user might use any of these methods to pre-process the spectra. The program includes one additional method to allow user defined pre-process functions. All methods iterate independently over the spectrum of each pixel. The wrapper for the user-defined function is no exception. Through the wrapper the user can pass either a lambda function or functions defined in other packages. By these means, the user can perform other common pre-processing steps such as applying a Savitzky-Golay filter (Jardim and Morgado-Dias, 2020) (see the R package ‘signal’ (Signal developers, 2014)).

Process. Currently the package implements only one algorithm to match pixel spectra with known references. It uses Spectral Angle Mapper (SAM) as implemented in the R package RStoolbox (Leutner et al., 2019). The algorithm recognizes different polymers successfully (Wu et al., 2020b), and to this end the waste recycling industry has used it for over 15 years (Kulcke et al., 2003). The SAM algorithm is, however, just one of the classical methods used for hyperspectral image classification. Researchers have proposed both algorithm optimizations (Galal et al., 2012; Tang et al., 2015) and alternative approaches (Kakhani and Mokhtarzade, 2019). The package modularity allows the user to add new processing algorithms by calling other R packages.

Post-process. The package implements two optional post-processing methods. The method to smooth the output of the SAM algorithm has the highest relevance. To smooth the image, the program uses a moving window to remove single-point particles. The approach improves pixel classification and facilitates other (manual) post-classification modifications (Galletti and Myint, 2014). The moving window algorithm has proven its worth in classification scenarios where researchers expect a great feature segmentation after the classification algorithm (Zheng et al., 2020). The method does not change the pixel resolution (size), it reassigns pixels values when they do not match their neighbours. The second post-processing tool implemented is a clipper or area selection-tool. Commonly, technicians prepare the samples over a round filter. Scanning the whole image will yield a square with unneeded borders. The clipper function discards unnecessary information and defines a particular area for both summary and check methods.

Check and summarize. To check the accuracy of the library search algorithm, it is possible to retrieve the spectra of pixels that matched a particular substance or cluster. We labelled the method get_profile. When processing mosaics, the method works only retrieving the pixel information of a user-defined chunk to avoid memory overload. The program defines also methods to plot for every step, to allow stepwise inspection of the process performance.

The summary method returns a three column table with information about the number of particles, their area, and the cluster or substance to which they correspond. The method vectorizes the image to identify features (plastic particles). It uses GDAL (GDAL/OGR contributors, 2020) to do so.

Output files. The summary method writes to disk two files. Both files are common extensions of Geographic Information System software. The summary method writes one (raster) using the R GDAL API (Bivand et al., 2019; Bivand et al., 2013), and the

other (vector) by implementing in R the C++ GDAL polygonise function (GDAL/OGR contributors, 2020).

3.3.3 Package components

uFTIR v0.1.1 includes the following major functions:

mosaic_info. Function to load basic image information to memory. It is intended for mosaics that will be processed in chunks. The user must pass a connection to Agilent's *.dmd file.

```
mosaic_info (dmdfile)
```

tile_read, mosaic_chunk. Functions to read a single tile or chunk to memory. The user must pass a connection to Agilent's *.bsp or *.dmd file. To load a chunk, it also needs the object returned by mosaic_info.

```
tile_read (bspfile)
mosaic_chunk (info, dmdfile)
```

preprocess. Lambda function wrapper to allow users to pre-process the spectra with user-defined algorithms.

```
preprocess (data, FUN)
```

wavealign. Function to resample a data set and a spectral reference library to a common extent. It uses the wavenumbers of data.x to sample data.y and then clips both to a common extent.

```
wavealign (data.x, data.y)
```

tile_sam. Function to perform the SAM algorithm for single tiles or mosaic chunks measurements. To do so it takes a SpectralPack object; the object returned by wavealign. It has an argument to indicate if derivatives of the spectra should be used instead of the raw spectra. The function returns a stack-like data type object. Each stack-slice holds a

match with one of the spectral references included in the spectral library. The top slice corresponds to the best match.

```
tile_sam (SpectralPack, derivative = NULL)
```

mosaic_sam. Function to perform the SAM algorithm for mosaics. As the program does not load mosaics to memory until they are processed —to avoid memory overload—, the function has arguments to pass instructions to pre-process the spectra before the matching. The function supports parallel computing.

```
mosaic_sam (info, spectral-reference, derivative = NULL,
        base_corr = TRUE, FUN = NULL, n_cores = NULL)
```

smooth_sam. Function to smooth the images returned by the SAM algorithm. It takes a user defined window size and it returns the stack-slices requested by the user.

```
smooth_sam (x, nclusters, window = 5, nslices = 1)
```

clipper. Function to clip the images to a given extent. The program applies the function only in a target stack-slice.

```
clipper (tarjet, centre = c(128, 128), rad = 120, slice = 1)
```

get_profile. Method to recover the spectra of all pixels that match a given substance. It can call different methods to plot internally. The x argument takes an object returned by mosaic_sam, tile_sam, smooth_sam, or clipper. The where argument takes the object returned by tile_read or a two item list that holds the object returned by mosaic_info and a connection to a *.dmd file. We made the difference to support the method for single tiles and mosaics, respectively.

```
get_profile (x, where, dst_cluster, plotpol = TRUE, plotpt = FALSE,
        cluster = TRUE, slice = 1, clusternames = NULL, …)
```

summary. Function to summarize the output of the SAM algorithm whether it was post-processed or not. It can call different post-processes internally. The function writes on disk two output files, that can be placed in a temporal directory.

```
summary (object, mask = NULL, clusternames = NULL,
        slice = 1, window = NULL, smooth = TRUE,
        temporal = FALSE)
```

plot. Methods to plot. It uses the R raster package (Hijmans, 2020). The … argument can be used to pass arguments to either raster::plot or graphics::plot.

```
plot (x, slice = 1, FUN = sum, …)
```

3.4 Algorithm Validation

Although the Spectral Mapper Algorithm discriminates well between polymers (Wu et al., 2020b) we tested whether it was correctly implemented in the uFTIR package. To do so, we recorded the spectra of one polyethylene bag, two plastic cups —one made of polypropylene and the other made of polystyrene—, and a polystyrene standard film (VARIAN P/N 883-9120). A single tile was recorded for each polymer, in transmission mode with a spectral resolution of 8 cm^{-1} through a spectral range of 3500 – 1300 cm^{-1} and 8 co-added scans. Data was recorded in absorbance (%). The microscope magnification was x4 with a pixel size resolution of 20.6 µm. The analysis used the spectra's first derivatives. The images post-process included smoothing them using a 3x3 moving window. We used a freely available spectral library for the library search (Primpke et al., 2018).

Results showed that the algorithm matches the expected polymer in all cases (Table 3.1). uFTIR classified correctly all pixels of the standard polystyrene film, and almost all pixels of the polystyrene cup. The algorithm was confused in 1% of the cases when it classified the polypropylene cup, attributing wrongly 88 pixels to polyethylene. The analysis of the polyethylene bag had the lowest success rate, misclassifying 4% of the pixels. However, the algorithm attributed those pixels to ethylene-vinyl-acetate, which is a polymer composed by polyethylene and vinyl-acetate in a ratio from 10:1 to 10:4. Figure 3.2 shows the average spectra recorded for each of the polymers used in the validation test and contrasts them with their reference spectra.

Table 3.1 *Polymers scanned and analysed in the validation test: number of particles detected, total area (pixel^2), proportion of the total area, other polymers identified in the same image, and the area of those other polymers (pixel^2).*

Polymer	Part.	Area		Other polymer types	Area of other polymers
	n	pixel^2	prop. area	—	pixel^2
polyethylene	2	15,705	0.96	ethylene-vinyl-acetate	679
polypropylene	1	16,296	0.99	polyethylene	88
polystyrene	1	16,351	>0.99	polypropylene	33
polystyrene standard	1	16,384	1	—	0

3.5 Illustrative Example

To illustrate the workflow of uFTIR and compare its output with its alternative (siMPle), we prepared a soil sample and captured its spectral signal (section 3.5.2). We processed the image using both uFTIR and siMPle software with similar settings (section 3.5.3). The software siMPle was developed to automate a similar analytical procedure. It implements a library search method, but it uses a correlation algorithm to perform the spectral matching. To produce comparable results, we used Primpke et al. (2018) library for both analyses. Section 3.5.4 presents the results of the analysis done with the uFTIR package, and section 3.5.5 shows the results produced using siMPle software.

3.5.1 Sample description

Three years ago the Chilean government assessed the fertility status of the country's soils (Corradini et al., 2019b). The service that carried out the laboratory analysis archived the soil samples. We took a small subsample of 10 samples from the archive and screen each of them looking for microplastics. We used the visual sorting method proposed by Zhang et al. (2018). After the screening, we kept one of the ten samples to use it in out illustrative example; the one with the highest content of microplastics. For this sample, the analysis reported 1.4 plastic particles per gram of soil.

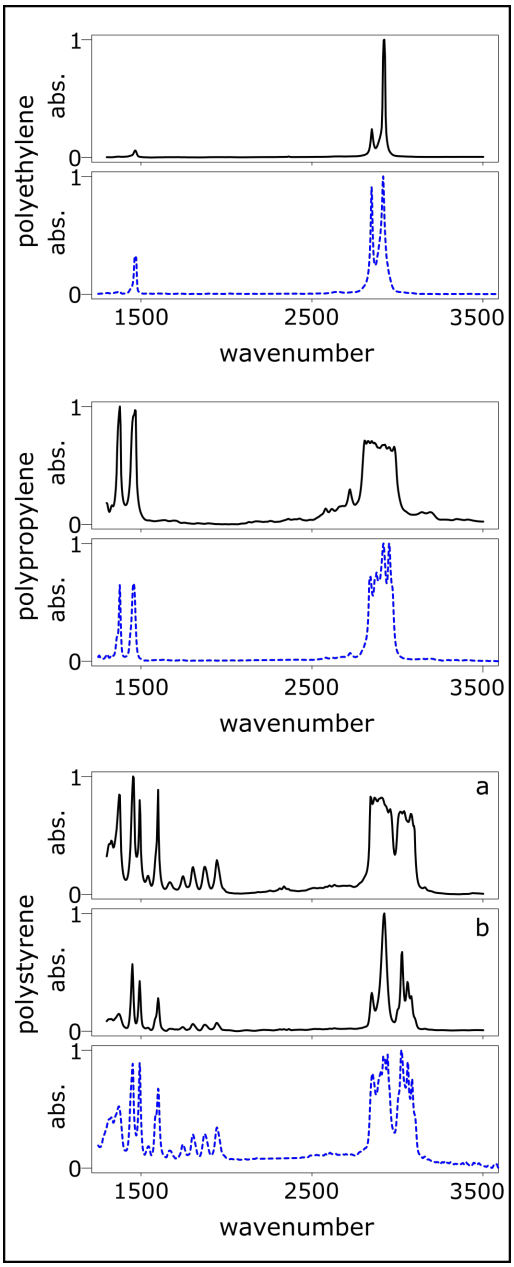

Figure 3.2 *Spectra recorded for each plastic polymer used to validate the matching algorithm. Black lines present the average spectra of all pixels that matched the target polymer while the blue-dotted lines show the spectral library's spectra for the target polymers. Polystyrene (a) corresponds to the plastic cup sample, while polystyrene (b) corresponds to the polystyrene standard.*

3.5.2 Sample preparation and image acquisition

The soil sample was suspended in $ZnCl_2$, stirred, centrifuged, and vacuum-filtered three times. At the end of the preparation process, a filter (Whatman(R) Anodisc Inorganic Membranes) that collected all buoyant particles was ready for µFTIR analysis. The µFTIR analysis was performed in transmission mode with a spectral resolution of 8 cm^{-1} through a spectral range of 3500 – 1300 cm^{-1} and 8 co-added scans. Data was recorded in absorbance (%). The microscope magnification was x4 with a pixel size resolution of 20.6 µm. The final mosaic comprised 64 tiles and 12Gb.

The collected image showed a large plastic particle placed on the filter's lower half. We opened the image in Agilent's Resolution Pro software and performed a library search in 10 random pixels within the particle. We used the correlation algorithm and the 'poly_8' built-in library. The particle matched polystyrene in all the 10 runs.

3.5.3 Hardware information

The image analysis with the uFTIR package was done in a Lenovo ThinkPad X220, Intel(R) Core(TM) i5-2540M CPU @ 2.60GHz, with 2 cores and 4 threads, and 8GiB of memory. The testing environment was Linux 4.19.0-8-amd64, Debian 10 and R version 3.5.2.

The image analysis with siMPle software (see section 3.5.5) was done in a HP EliteBook 840-g3, Intel(R) Core(TM) i7-6600U CPU @ 2.60GHz, with 2 cores and 4 threads, and 8GiB of memory. The testing environment was Windows 10 enterprise, with siMPle Version 1.0.0.

3.5.4 uFTIR pre-processing and results

The image was processed as mosaic using the package parallel features. The pre-process included scaling and taking the spectra first derivatives. The post-process included smoothing the image with a moving window of 3x3 pixels and clipping it to the extent of the filter to leave out the filter's polypropylene support ring. The clipping mask was a circle with a radius equal to 490px and its centre placed at (512,512)px. Figure 3.3 shows the output of each analytical step.

The analysis took 8min 20s to complete (elapsed time).

Table 3.2 *Summary of uFTIR's analysis.*

Cluster name	Number of particles	Total area
	n	pixel^2
animal fur	155	60,877
chitin	41	1,109
coal	482	239,974
plant fibres	433	415,434
polypropylene	48	791
polystyrene	3	5,224

The analysis revealed the presence of two different polymer clusters on the filter. Polystyrene dominated with 3 particles that accounted for more than 5,000 pixels^2. Polypropylene was the other, having 48 particles and a total area of ~800 pixels^2. Table 3.2 reports the summarized output. Figure 3.3(f) shows the correspondence between the library spectra (blue-dotted) and the average spectrum of the 3 particles that matched polystyrene (red-solid). The polystyrene particle was fragmented into three particles. However, two of them had an area of 2pixels^2 and one encompassed >99% of the particle area.

3.5.5 siMPle pre-processing and results

The pre-process included cutting out the CO_2 signal and taking the spectra first derivatives. We exported siMPle results as comma delimited to summarize them in R. Figure 3.4 shows the image output and a close-up to the large polystyrene particle.

The analysis revealed the presence of 18 synthetic polymers. Table 3.3 shows a synthesis of the output. The polystyrene particle matched both polyimide and polysulfone (and not polystyrene). siMPle identified only 3 polystyrene particles, with a total area of 9 pixels. The large number of particles for each cluster revealed a problem of particle fractionation. The large amount of polypropylene corresponds to the filter's support ring (see Fig 3.4(c)). The program has no features to crop, or smooth the output.

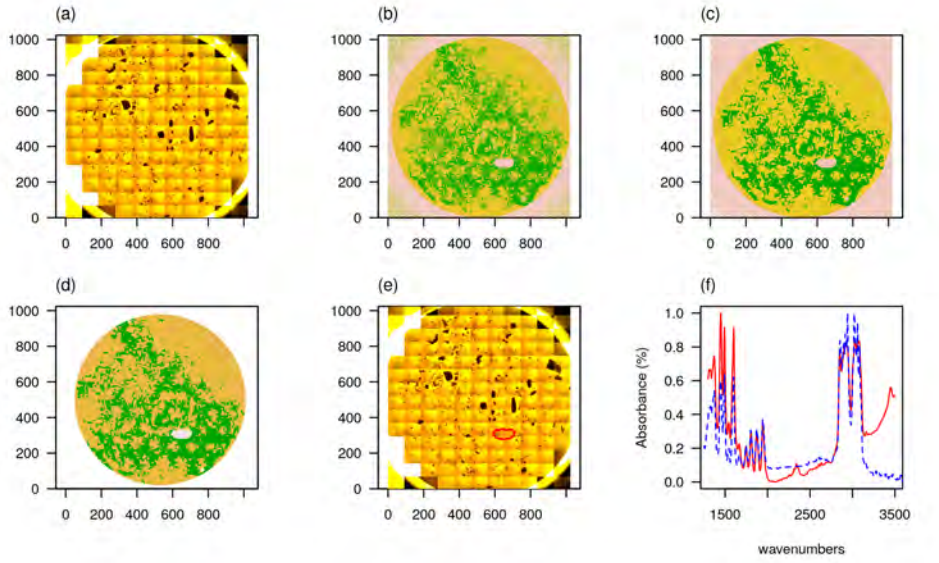

Figure 3.3 *uFTIR workflow and analysis at different steps for an environmental sample: (a) load the sample (visual) image into memory, (b) pre-process the spectra and run the library search algorithm (spectral angle mapper), (c) post-process the image with a smoother algorithm (moving window), (d) post-process the image removing unnecessary information (clip), (e) check the accuracy by tracing a polygon over all particles matching a given polymer (polystyrene), (f) check the accuracy by comparing the spectral signal (mean) of all particles that matched a given polymer (polystyrene - red-solid line) and the reference spectra of the polymer (polystyrene in Primpke et al. (2018)'s library — blue-dotted line).*

The software took 50s to convert the image to siMPle's format, 18s to load the reference library, 46min to analyse the image for spectra fit, and 3h 48min 9s to run the 'MP detection' algorithm to find the particles. The total time was 275 min.

3.6 Impact

The uFTIR package provides a general-purpose software to automatize hyperspectral images acquired in µFTIR spectrometers. Its primary orientation is towards microplastic detection. It constitutes a step forward for environmental research as it provides a tool for researchers to increase the accuracy of state-of-the-art analytical methods. Complete automation of microplastic detection in soil samples is a milestone yet to be accomplished.

The software, however, narrows the gap by providing a tool that implements a scalable methodology —in a language familiar to scientists— that quantifies and identifies microplastics in environmental samples.

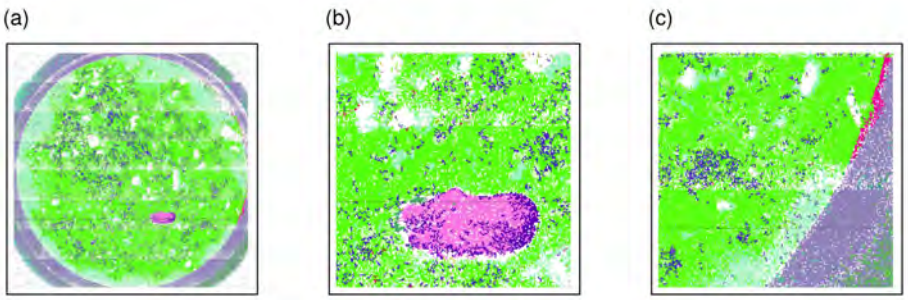

Figure 3.4 *Output of siMPle: (a) image map after the 'MP detection', (b) close-up to the polystyrene particle, revealing the particle fractionation problem, (c) close-up to the filter support ring that matched polypropylene.*

We present a software that implements library search using spectral angle mapper. The algorithm had not been implemented before in any similar software (such as siMPle (Primpke et al., 2019a) or MPhunter (Primpke et al., 2017)), and comes back to the idea of exporting earth science tools to spectral microscopy (Harris, 2006).

The algorithm and the analytical workflow implemented in uFTIR allows the package to work several orders of magnitude faster than its alternative (siMPle, see section 3.5). The increase in speed will allow researchers to increase the number of samples in assessment efforts. This will contribute to size the problem of plastic pollution in ecosystems without the current limitations imposed by time consuming and tedious laboratory routines (Jany et al., 2020).

The package improves the reproducibility of the results, since procedural scripts can be shared and published together with scientific articles. The software open-source nature allows trustworthy analysis and scientific communication (Chambers, 2008). Moreover, R —a functional programming language— is strongly modular, facilitating the addition of new functions and analytical techniques. The language is also common among scientists, a fact that should impact on the software placement and adoption.

In its first release, the package implements only one matching algorithm. However, the R environment is full of packages that can interact in any of the analytical steps described. As a proof of concept, we show an example that uses the R 'signal' package (Signal developers,

2014) to include a Savitzky-Golay filter to pre-process a sample in the CodeOcean computer capsule that accompanies the original publication of this chapter (Corradini, 2020). In future releases, we expect to include a support vector machine algorithm, another hyperspectral image classification method with good reputation among scientists (Kakhani and Mokhtarzade, 2019).

Table 3.3 *Abbreviated summary of siMPle's analysis.*

Cluster name	Number of particles	Total area
	n	pixel^2
polypropylene	26,633	1,214,800
not identified	14,516	608,093
cellulose	2,793	27,192
acrylates/PUR/varnish	905	3,249
polyimide	16	3,046
polyethylene	878	2,864
polyester	600	1,940
polysulfone	61	1,142
polycaprolactone	286	974
plant fibres	108	447
⋮	⋮	⋮
animal	3	10
polystyrene	3	9

3.7 Conclusion

We presented uFTIR, an R-based software that implements an automatic approach to analyse µFTIR images. The package is mainly oriented towards the analysis of environmental samples and microplastic identification. It supports parallel computations, and interaction with other R packages and procedures. It is fast, compared with other library search alternatives, and it promotes trustworthy science through an open-source approach. uFTIR is an ongoing project. We intend to implement additional matching algorithms in future releases, and a pre-processing feature for *a priori* feature recognition. As presented, we hope that our contribution will serve researchers to size the occurrence of microplastics in ecosystems.

Chapter 4. Evidence of microplastic accumulation in agricultural soils from sewage sludge disposal

Microplastics are emerging as a steadily increasing environmental threat. Wastewater treatment plants efficiently remove microplastics from sewage, trapping the particles in the sludge and preventing their entrance into aquatic environments. Treatment plants are essentially taking the microplastics out of the waste water and concentrating them in the sludge, however. It has become common practice to use this sludge on agricultural soils as a fertilizer. The aim of the current research was to evaluate the microplastic contamination of soils by this practice, assessing the implications of successive sludge applications by looking at the total count of microplastic particles in soil samples. Thirty-one agricultural fields with different sludge application records and similar edaphoclimatic conditions were evaluated. Field records of sludge application covered a ten year period. For all fields, historical disposal events used the same amount of sludge (40 ton ha^{-1} dry weight). Extraction of microplastics was done by flotation and particles were then counted and classified with the help of a microscope. Seven sludge samples were collected in the fields that underwent sludge applications during the study period. Soils where 1, 2, 3, 4, and 5 applications of sludge had been performed had a median of 1.1, 1.6, 1.7, 2.3, and 3.5 particles g^{-1} dry soil, respectively. There were statistical differences in the microplastic contents related to the number of applications that a field had undergone (1, 2 , 3 < 4, 5). Microplastic content in sludge ranged from 18 to 41 particles g^{-1}, with a median of 34 particles g^{-1}. The majority of the observed microplastics were fibers (90% in sludge, and 97% in soil). Our results indicate that microplastic counts increase over time where successive sludge applications are performed. Microplastics observed in soil samples stress the relevance of sludge as a driver of soil microplastic contamination.

Based on:

Corradini, F, P Meza, R Eguiluz, F Casado, E Huerta-Lwanga, V Geissen. 2019. Evidence of microplastics accumulation in agricultural soils from sewage sludge disposal. Science of the Total Environment 671:411-420.

4.1 Introduction

Human activities are directly responsible for aquatic and terrestrial microplastic contamination. In recent years, more research has been performed to assess the different sources of microplastics and their relative impact on the environment (Auta et al., 2017; Bläsing and Amelung, 2018; Da Costa et al., 2019; Ng et al., 2018). Historically, researchers have mainly focused on examining the effects of plastic contamination arising from general littering, plastic waste dumping, and inappropriate management of landfill sites (Duis and Coors, 2016). However, over the last few years, this focus has grown to include environmental concerns arising from techniques used in the agricultural sector. The common agricultural practices of disposing of plastic mulching, water pipes, and plastic greenhouse covers have begun to raise concerns (Brodhagen et al., 2017; Steinmetz et al., 2016; Zhang and Liu, 2018). Although there is evidence supporting the fact that wastewater sludge used as a soil amendment could also be contributing to soil contamination (Zubris and Richards, 2005), a field evaluation further examining the effects of this practice has not yet been carried out.

Wastewater is a main source of microplastic contamination in freshwater environments. Wastewater is capable of transporting plastics from many different sources. Horton et al. (2017) observed that storm drains in the UK , for example, carry considerable amounts of synthetic fibers. In fact, synthetic fibers are a major source of microplastics in sewage (Henry et al., 2019; Ziajahromi et al., 2017). Fibers from textile materials originating from domestic washing machines have the potential of reaching aquatic environments even after sewage treatment (Hernandez et al., 2017; Napper and Thompson, 2016). Despite their relevance, they are not the only source of microplastics in sewage. Personal care products are also believed to contribute to microplastic pollution. Some brands of toothpaste, soaps and facial scrubs contain microplastics which could potentially reach aquatic environments through wastewater treatment plants (Napper et al., 2015). However, the contribution of these personal care has caused some scientific controversy (Duis and Coors, 2016).

The presence of microplastics in wastewater has been studied by several scientists who have come to the common agreement that overall, waste water treatment plants (WWTP) are efficiently removing microplastics from wastewater (Sun et al., 2019). This conclusion is great for aquatic environments where the wastewater eventually ends up. WWTPs effectively remove nearly 99% of microplastics from wastewater. This begs the question: Where do the microplastics go? Unfortunately, although the removal process benefits aquatic environments, the soil environment is less fortunate. Microplastics accumulate in the sludge produced in WWTPs (Li et al., 2018). This sludge is in turn used as fertilizer on agricultural fields. This practice has a positive impact on soil fertility and is still essential in

many countries all over the world (Coors et al., 2016; Schmidt et al., 2006). However, this use of sludge creates a pathway for microplastics to enter agricultural soils (Zubris and Richards, 2005).

Before making conjectures, several information gaps need to be addressed. Evidence supports the finding that synthetic fibers accumulate in soils treated with sludge (Zubris and Richards, 2005). We also know that plastic debris is found in sewage sludge (Li et al., 2018; Mahon et al., 2017). To our knowledge, there are no studies that evaluate the effect of successive sludge applications on agricultural fields. Therefore, with this research, we wanted to answer the question: Are microplastics accumulating in agricultural soils as a result of sewage sludge applications? There were two aims of this study: (1) to evaluate microplastic contamination in soils from sewage sludge applications and (2) to evaluate the impact of repeated applications of sewage sludge by examining soil samples. We did this by selecting and evaluating 31 fields in the Chilean central valley with different sludge application records which covered a period of ten years of sludge application.

4.2 Material and methods

4.2.1 Study site

Chile was chosen for this case study since the country has a ten year record of sludge applications (MINSEGPRES, 2009). Sludge disposal on agricultural fields has been permitted by local authorities in Chile since 2009. Sludge producers have to report each sludge application to the Chilean Agricultural and Livestock Service, which keeps a record of each application. We looked at all of the places were sludge has been applied over the last ten years. From this data, a hot spot area was selected which we hoped would prevent soil covariates from increasing experimental noise. The area was located in Mellipilla county, in the Metropolitana region of Chile. Within this county, a 10 km^2 area near the Maipo river was selected since it included 30 fields that were successively treated with sludge over the past 10 years. In the same area, we selected one control site where no sludge had been applied.

While all fields shared similar soil chemical and physical characteristics, they were exposed to different sludge application rates over time. All of the fields that were selected had a soil classified within the USDA Entic Haploxerolls subgroup (CIREN, 1996), a medium (loam) to moderately coarse (sandy loam) soil texture, a flat surface (0 - 1%), and an average depth of 75 to 100 cm. The soil organic matter (SOM) ranged from 1.3% to 4.3% (median = 2.1%). The fields, however, comprised different soil map units since they presented different degrees of stoniness. Regarding sludge applications, there were fields that were treated

with sludge up to five times, while others received only one application. The number of applications, the year when the last application was performed, and the crops produced after the application are shown for each field in Fig. 4.1. In nearly all fields, sludge was applied for the last time in 2017 and corn -either as a monoculture or in rotation- was the main crop after this last application. Hence, the only factor that significantly varied between fields was the number of sludge applications. The sludge applied was homogeneous. It originated from the same wastewater treatment plant and was stabilized before each application by solar desiccation or centrifugation.

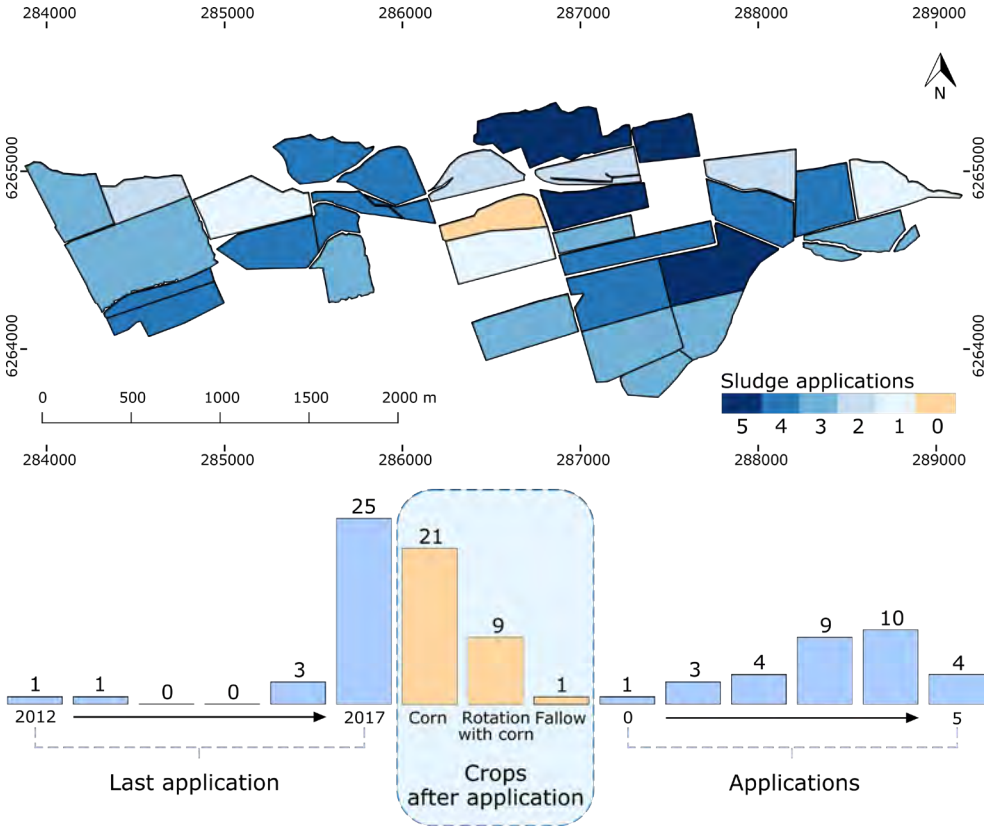

Figure 4.1 *Location of fields under study. The differences in the number of sludge applications carried out over time have been highlighted for each field (above). Quantity of fields (n) that: were applied until a given year (bottom-left); presented corn, a rotation with corn, or laid fallow after the last application (bottom-center), and; underwent a given number of sludge applications (bottom-right) (each application = 40 ton ha^{-1}).*

Current Chilean regulation allows no more than one application of 90 ton ha^{-1} of dewatered sludge per year per field (dry weight basis). This rate is almost never reached in practice since applying 90 ton ha^{-1} of dewatered sludge entails several technical problems. Therefore, on average, 40 ton ha^{-1} of dewatered sludge is applied to fields during each application (dry weight basis).

4.2.2 Sampling and sample preparation

Three soil samples, chosen randomly within each field boundary, were collected for each field. Each sample was taken from the topsoil (0 - 25 cm) using a metallic soil auger. The sampling depth corresponded with the reported depth of sludge application. Samples were transported in polypropylene (PP) plastic bags (3 mm thick) to the laboratory, where they were unpacked, spread over wood trays, and dried in an forced air oven at 40 ± 2 °C. The samples were then sieved with a <2 mm metal mesh and stored in polyethylene terephthalate (PET) jars. When present, microplastic particles larger than 2mm were counted by hand and noted.

A control sample without plastic was made to check whether the plastic containers polluted the samples with plastic, compromising the quality of the analysis. An agricultural soil was collected, dried, sieved, and ignited at 500 °C for 3 hours. The temperature reached ensured the elimination of all plastic particles (Anuar Sharuddin et al., 2017). After ignition, three replicates (500 g) of the ignited soil were placed in PP plastic bags, and shaken at 180 strokes min^{-1} for 10 minutes in a platform shaker to simulate transport. Samples were then unpacked and stored in PET jars. Since it was not possible to guarantee a site with no plastic contamination, no blanks were collected in the field or used during the handling procedure.

Seven sludge samples were collected on location, as some of the fields underwent sludge applications during 2018 (not considered in the study, as soil sampling was done before). The sludge samples were transported in plastic bags and then dried in an forced air oven at 40 ± 2 °C. Since the dried sludge formed hard clods, the samples were milled with a porcelain mortar and sieved using a <1 mm sieve, before being stored in PET containers. When present, microplastic particles larger than 2mm were counted by hand.

4.2.3 Laboratory analysis

There is no standardized procedure to quantify microplastics in soil samples. Therefore, we implemented a methodology based on two recent studies (Hurley et al., 2018; Zhang et al., 2018). The method takes advantage of the fact that plastics have a lower density than soil particles. We used a wet extraction technique to float the plastic particles. A general overview of the methodology is shown in Fig. 4.2.

The dried soil samples were weighed and 5 ± 0.01 g was placed in 50 ml glass centrifuge tubes. 20 ml of deionized water was added to each tube and the samples were then stirred at ~21,000 rpm for 30 seconds. A Dremel® 3000 (Robert Bosch Tool Corporation, IL, USA) with a custom-made rod and mixer palette was made to stir the samples (rod Ø = 3.2 mm, palette width = 5 mm). The high speed used allowed the soil to be completely suspended in our experimental set up. Samples were centrifuged at 2,000 rpm (2240 g) for 15 min and the supernatant was filtered using a Whatman No. 42 filter paper (retention >8 µm). 20 ml of sodium chloride (NaCl) 5M (ρ = 1.20 g cm^{-3}) was added to the precipitate, which was then stirred and centrifuged a second time. The supernatant was once again filtered through the same Whatman No. 42 filter paper or through a new one if the first one became clogged. If the filter was replaced, the first filter was saved in a Petri dish for optical inspection. 20 ml of a concentrated zinc chloride solution ($ZnCl_2$ 5M, ρ = 1.55 g cm^{-3}) was added to the centrifuge tubes with the precipitate for one final extraction. Since the $ZnCl_2$ solution had a higher viscosity than the previous solutions, the samples were stirred at 32,000 rpm for 30s. Centrifugation was carried out at 2,000 rpm (2240 g) for 15 minutes and the supernatant was filtered through Whatman No. 42 filter paper, while taking into account the replace-if-clogged indication. Filter papers were then saved in Petri dishes for optical inspection.

After microplastic extraction, the filters were inspected using a stereo microscope (model SMZ 745T coupled with a NI-150 high intensity illuminator, Nikon, Tokyo, Japan) at 20x. The microplastic particles collected on each filter were counted twice. Microplastic particles were considered to have shiny surfaces, strong colors, and sharp geometrical shapes. Synthetic fibers were considered to have smooth sides and strong colors, as laid out by Horton et al. (2017). Particles were classified according to their shapes: fibers, fragments (angular and solid), films (flexible and thin), or pellets (rounded and solid). Together, fragments, films, and pellets are referred to as non-fiber particles. A random sample of the examined microplastics were photographed (Micrometrics® camera model 519CU CMOS 5.0 Megapixel, ACCU-SCOPE Inc., NY, USA) to measure the length and width if fibers and the surface area for non-fiber particles. ImageJ 1.5 software was used for this purpose (Schneider et al., 2012). Results were reported as number of microplastic particles per 5 g of dry soil (p $5g^{-1}$).

An estimation of the weight of the microplastics was performed using the measured area (Simon et al., 2018). The fiber weight was calculated using the width as the fiber diameter while considering a 40% of void fraction. The area of pellets was measured within a circle to estimate the volume of a perfect sphere. Fragment volumes were approximated using ellipsoids. A thickness of 13 µm was used to calculate film volumes. The weight was estimated using a density of 1.38 g cm^{-3} for fibers (polyester) and 1.35 g cm^{-3} for non-fibers (polyvinyl chloride).

Sludge samples were analyzed in a similar way but microplastic counts turned out to be too high in sludge samples so only 1 g of sludge was per tube was measured.

As a quality control measure, each set of samples (n = 20) included one reagent blank. The filter from this blank was saved in a Petri dish and inspected at the end of the analysis. The measurement should have accounted not only for the quality of the reagents used but also for any contamination inside the lab (Mahon et al., 2017; Schneider et al., 2012). All materials used in the sample analysis were made of glass (funnels, Petri dishes, centrifuge tubes) and the stirrer rod was made of stainless steel. White cotton lab coats were used by the analysts during analysis and sample manipulation.

4.2.4 Method validation

In order to test our method, 10 soil samples from the region were selected for the validation experiments. Microplastics were then added to the soil samples which were then put through the same treatment as the rest of our soil samples. This allowed us to check the recovery rates of the added microplastics. The selected soils had between 1.0 and 4.0% SOM and were 12 to 44% clay, with textural classes ranging from clay loam to sandy loam.

Acrylic, polyester, and nylon fibers, as well as low density polyethylene and polyvinyl chloride particles were used to pollute the samples. Each of these polymers were sourced using a different method. A ball of acrylic wool was cut into pieces and the acrylic threads were cut with an electric hair cutter. Ready-made polyester fibers normally used as cushion stuffing were purchased and cut with scissors into shorter lengths. A pair of pantyhose (98% nylon - 2% elastane) was processed as an acrylic sample since the elastane compound was present only in the waist support, which was discarded before preparing the fabric for the samples. Low density polyethylene (LDPE) particles were obtained by freezing and milling LDPE pellets (SABIC® LDPE). Lastly, a polyvinyl chloride (PVC) water poncho was cut and rasped with a rectangular rasp. After size reduction, all polymer particles were sieved using a >2 mm mesh. A sample of each polymer was photographed under the microscope and their dimensions were measured using ImageJ 1.5 (Schneider et al., 2012). The size of the polymers used in the recovery test are shown in Table 4.1.

Three fibers or particles of each polymer were included in the microplastic sample that was used in the validation of the method. 5 g of the soil samples were put into centrifuge tubes along with 3 fibers/particles of each polymer. 10 ml of distilled water was added to the prepared samples, which were then mixed with a glass stirring rod and allowed to dry. Samples were wetted and air dried twice more in order to emulate natural wetting and drying cycles, as suggested by (Hurley et al., 2018). Three replicates per soil sample were analyzed. Recovery was expressed as a proportion of observed polymers after the

extraction process. The recovery rate from the first two solutions (H_2O and NaCl 5M) was registered separately from the third ($ZnCl_2$ 5M) and two filter papers were used per extraction.

Along with this recovery assessment, the method repeatability was evaluated. Five random samples were analyzed a total of five times.

Table 4.1 *Average particle size by polymer type used in the validation set and their standard deviation.*

Polymer	Shape	Length	Width	Area
		mm	mm	mm^2
Acrylic	**Fiber**	**2.7 ± 1.4**	**0.04 ± 0.01**	**0.12 ± 0.06**
Polyester	**Fiber**	**1.60 ± 1.1**	**0.04 ± 0.01**	**0.07 ± 0.06**
Nylon	**Fiber**	**2.30 ± 0.8**	**0.05 ± 0.01**	**0.98 ± 0.37**
LDPE	**Fragment**	-	-	**0.16 ± 0.10**
PVC	**Fragment**	-	-	**0.10 ± 0.08**

4.2.5 Statistical analysis

4.2.5.1 Method validation

The data from the validation procedure was analyzed using a split-plot design where the whole plots corresponded to the SOM and the split-plots to the plastic polymer. Total recovery was analyzed. In order to look for significant differences, a logistic regression model was fitted to data and an ANOVA test was performed using a significance >95%. A logistic regression was used since the recovery rate corresponded directly to the added polymers recovered. The overall effect of SOM and the hypothesis which focused on finding differences between each plastic polymer were tested using the Wald chi-squared test (R Package **A**nalysis of **O**verdispersed **D**ata 'aod', Lesnoff and Lancelot (2012)).

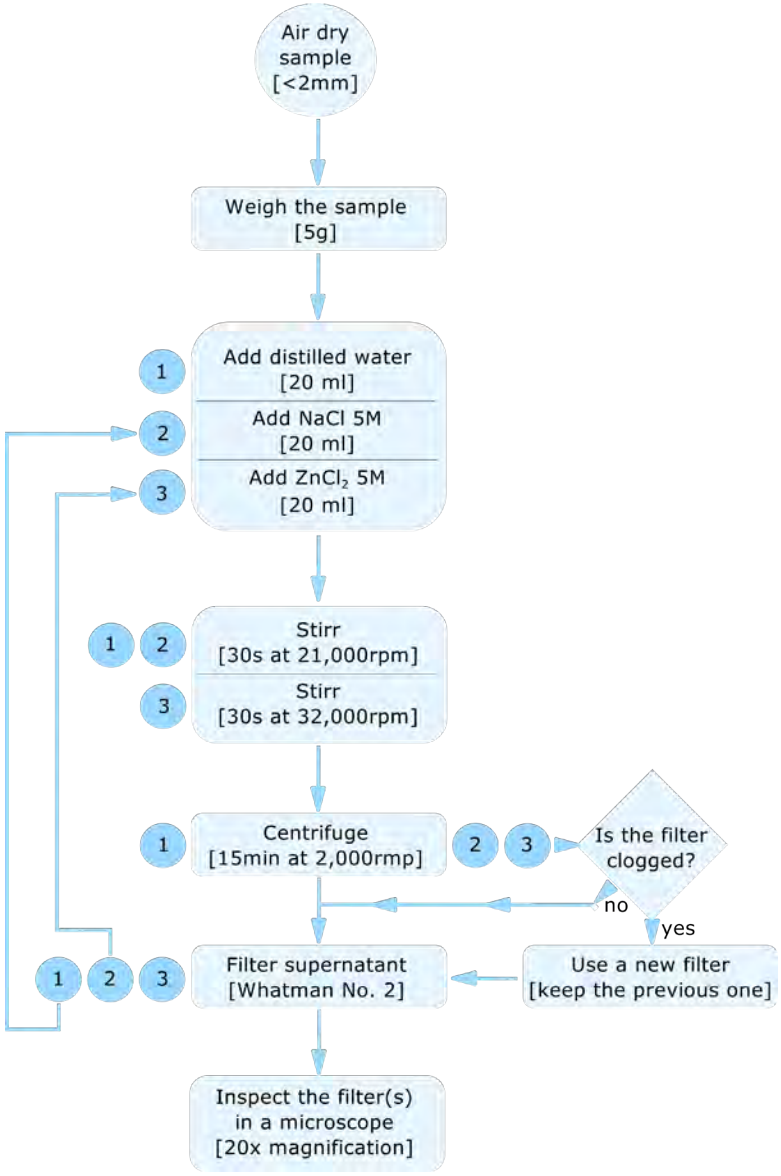

Figure 4.2 *Diagram of the method used. Samples were dried, sieved and weighted. Different solutions were added in sequential time steps to extract the plastics by flotation. First water was used ① then the sample was stirred, centrifuged, and filtered. Next, a solution of NaCl was used ② and the sample is again stirred, centrifuged, and filtered. The first filter was replaced only if it was clogged. Thirdly, a solution of $ZnCl_2$ was used ③ then the sample was stirred, centrifuged, and filtered for the last time. The previous filter was replaced only if it was clogged. The filters were then inspected in a microscope to identify plastic particles.*

The microplastic counts from the repeatability trial were analyzed after grouping the data by sample. The average, standard deviation, and coefficient of variation were calculated and the repeatability reported as the overall mean of the standard deviation and the coefficient of variation.

4.2.5.2 Soil microplastic contamination

The results from the 3 samples taken from each field were averaged. The difference in soil microplastic counts was evaluated as a function of the quantity of sludge applied. The microplastic count was the dependent variable, while the number of sludge applications was the independent variable. In this way, the number of sludge applications was set as the treatment with five levels (1, 2, 3, 4, and 5 sludge applications). An ANOVA test was performed to look for statistical differences. Counts were transformed using the natural logarithm to satisfy ANOVA assumptions. Significant differences were considered to occur when a significance >95% was observed. Fisher's last significant different test (LSD) was used to compare results between treatments (number of sludge applications).

Since there was only one control site (no replicates), zero sludge applications was not considered as a level of the independent variable. Instead, the Wilcoxon signed rank test was used to check whether or not the mean of the control site was different from 0. The microplastic counts of each treatment (number of sludge applications) were compared with the mean of the control site by a one-sample Student's t test after the data were transformed using the natural logarithm.

The fiber to microplastic ratio was calculated as the number of fibers divided by the total microplastic count. The ratio was defined as the dependent variable with the sample matrix (sludge or soil) as the independent variable. An ANOVA model was fitted to the data to look for significant differences in the proportion of fibers with respect to the total count of plastics between sludge and soil samples. Differences were evaluated for a 95% confidence interval (CI).

Descriptive statistics were used to characterize sludge and soil microplastic content, fiber length and width and non-fiber-particle surface area.

All statistical analysis were performed in the R environment (R Core Team, 2020).

4.2.6 Method validation

Three sequential extractions were enough to get high recovery rates. All plastic polymers used presented statistically different recovery rates, which were influenced by SOM (Fig. 4.3). The recoveries observed were LDPE > Polyester > PVC > Nylon > Acrylic. Only acrylic

fibers presented a poor recovery (mean = 49%) while other polymers showed a relatively high recovery rate (>77%). The recovery rate of LDPE particles stood out with 98% of the particles being recovered. In all cases, recovery was increased as a result of a third extraction carried out with $ZnCl_2$. This was especially true for PVC. Although soil organic matter did affect the recovery rate of each polymer, this effect was considered negligible for the study area.

The repeatability was acceptable, with a standard deviation of 1.5 p $5g^{-1}$ and a coefficient of variation of 12% in samples that ranged between 8 and 20 p $5g^{-1}$. With regard to packaging/transport contamination, only 1 fiber was found in one of the three ignited soil samples. It was a blue fiber, which did not match the color of the PP plastic bags or the PET jars. Although the effects of the packaging was negligible, the use of plastic bags should be avoided whenever possible. Around 33% of reagent blanks were polluted with 1 fiber, while the remaining 66% had a null count. The analysis of a set of 20 samples -including a reagent blank- took a day (~7.5 hours).

4.3 Results

4.3.1 Microplastics in soil samples

Overall, with each successive sludge application, there was an increase in the median (Fig. 4.4). The control site had the lowest microplastic count. Two of the three samples from the control site scored 1 p $5g^{-1}$, while the other had 3 p $5g^{-1}$. The mean of the control was different from zero (p-value = 0.08). The means of the treatments were different from the control mean (p-value <0.01 for 5, 4, 3, and 2 applications, and p-value = 0.05 for 1 application). One, two, and three sludge applications had a similar effect with regards to microplastic accumulation in the topsoil. There were no differences between the means of 2, 4, and 5 sludge applications. The data dispersion was the highest for 3 sludge applications, where data ranged from 2.3 to 19 p $5g^{-1}$. Sludge presented a high microplastic content (median = 170 p $5g^{-1}$), which stood out from the soil observations. The weight estimates are presented in Table 4.2.

4.3.2 Microplastic characterization

The majority of microplastics observed in both soils and sludge samples were fibers. While the mean fiber to microplastic ratio for sludge was 0.90 ± 0.05, the proportion of fibers for soil samples was 0.97 ± 0.03. The ANOVA result indicates that the fiber to microplastic ratio was statistically different between soils and sludge samples. Most of the fibers observed were small, with only a few fibers having a width >50 µm (Fig. 4.5a). The median fiber width

was 20 µm, while the interquartile range (IQR) was 10 µm (p.25 = 17 µm and p.75 = 27 µm). The fiber length showed a similar distribution, having a median of 0.97 mm and a IQR of 1.05 mm (p.25 = 0.62 mm and p.75 = 1.67 mm) (Fig. 4.5b). Twenty percent of the fibers observed had a length >2 mm, and only 5% had a length >4 mm. The shortest fiber observed was 0.16 mm, and the narrowest was 8 µm.

Non-fiber particle shapes were predominantly films (58%) and a particle's surface area was generally <0.5 mm^2 (Fig. 4.6). The median for the particle surface area was 0.03 mm^2 and the IQR was 0.12 mm^2 (p.25 = 0.01 mm^2 and p.75 = 0.13 mm^2). The smallest particle observed was 0.0023 mm^2 (2254 μm^2). Example images of the observed microplastics are provided in Fig. 4.7.

4.4 Discussion

4.4.1 Accumulation of microplastics in soils

Our evidence suggests that microplastics accumulate in soils with successive sludge applications. While the presence of synthetic fibers in sewage sludge has been known since the end of the 90's (Habib et al., 1998), their accumulation in soil by sludge disposal was first acknowledge almost ten years later (Zubris and Richards, 2005). These authors reported a mean count of 1.21 ± 0.25 synthetic fibers per g^{-1} of soil five years after dewatered sludge was applied to soil columns for the last time. While the amount of sludge applied in Zubris and Richards' study was high (215 dry ton ha^{-1} to simulate 30 years of agronomic applications), the amount of sludge that is usually applied on Chilean fields are also exceptionally high (200 dry tons ha^{-1} can be reached within five years). In our study, fields where sludge had been deposited 5 times (200 dry tons ha^{-1}) had an median of 3.5 p g^{-1} soil one year after application, which is almost three times the amount reported by (Zubris and Richards, 2005). To complement the experiment of the soil column, Zubris and Richards evaluated a soil amended with 300 ton ha^{-1} of alkaline-stabilized sludge two years after application, reporting ~2.5 synthetic fibers g^{-1} soil (topsoil). As the alkaline-stabilization process abrades synthetic fibers, this result can be considered similar to ours.

Despite the evidence of synthetic fibers accumulating in soils after sludge application and the growing concern surrounding plastic contamination of soils, there are only a few recent studies evaluating plastic accumulation in agricultural soils (Da Costa et al., 2019). Liu et al. (2018) reported that farmland soils near Shanghai in China had a maximum microplastic content of 0.28 p g^{-1} soil, with an average of 0.078 ± 0.013 p g^{-1} in the topsoil. Although these authors pointed to sludge as a possible source of the observed microplastics, they were unable to report on application rates. A different study in China evaluated the

microplastic content in soils under intensive agriculture (Zhang and Liu, 2018). The authors reported that soils where approximately 23 tons ha^{-1} $year^{-1}$ of sludge were applied had between 7 and 43 p g^{-1} of microplastics at 0 to 10 cm depth. The reported range is by far greater than ours, but the authors had additional sources of microplastics polluting the soil such as plastic mulch and other plastic covers, which were not included in our study area. Nonetheless, the authors found that the majority of microplastics were fibers (92%), which match our findings.

Other studies that reported expected microplastic concentrations in soils did not evaluate agricultural soils with sludge applications. While they are not relevant for comparison, they emphasize the relevance of the sludge contribution to microplastic soil contamination. Home gardens in rural Mexico, where plastic household waste accumulates, averaged 0.9 ± 1.9 p g^{-1} soil (Huerta Lwanga et al., 2017b). An agricultural field in China, where plastic mulch is used to reduce soil water evaporation, averaged 0.10 ± 0.14 p g^{-1} soil (Zhang et al., 2018). As previously mentioned, data concerning plastic soil contamination is scarce, but if the average reported by Zhang et al. (2018) is taken as a reference and compared to the data in Table 4.2, sludge can contribute 101 times more plastic particles in a year than plastic mulching.

Other studies reporting microplastic content in sewage sludge present slightly lower results. An extensive survey of microplastic contamination of sewage sludge in China reported an average of 22.7 ± 12.1 p g^{-1}, from which 63% were fibers (Liu et al., 2018). In Ireland, where sewage sludge undergoes different stabilization treatments, researchers found microplastic concentrations that ranged from 4.1 to 15.4 p g^{-1} (Mahon et al., 2017). When they evaluated a composted sludge, they found that 91% of the particles were fibers, which match our findings. At the study site, sludge underwent a simple process before disposal (centrifugation or solar desiccation), which could explain the higher microplastic counts observed.

An explanation for the fact that the control site had between 1 and 3 p g^{-1} soil can be difficult to confirm. On the one hand, it has been reported that microplastics can reach remote places by aeolian transport processes that are only partially understood (Prata, 2018). In Switzerland, a country with a successful plastic waste management strategy, researchers found evidence of aeolian and fluvial deposition of plastic in rural areas far from any polluting source (Scheurer and Bigalke, 2018). Generally speaking, the ubiquity of microplastics in agricultural environments makes it difficult to find a control site where absolutely no plastic is present. This was the case for studies performed in China (Zhang and Liu, 2018) and in the USA (Zubris and Richards, 2005). On the other hand, contamination could occur during the sample collection and handling (section 4.4.2).

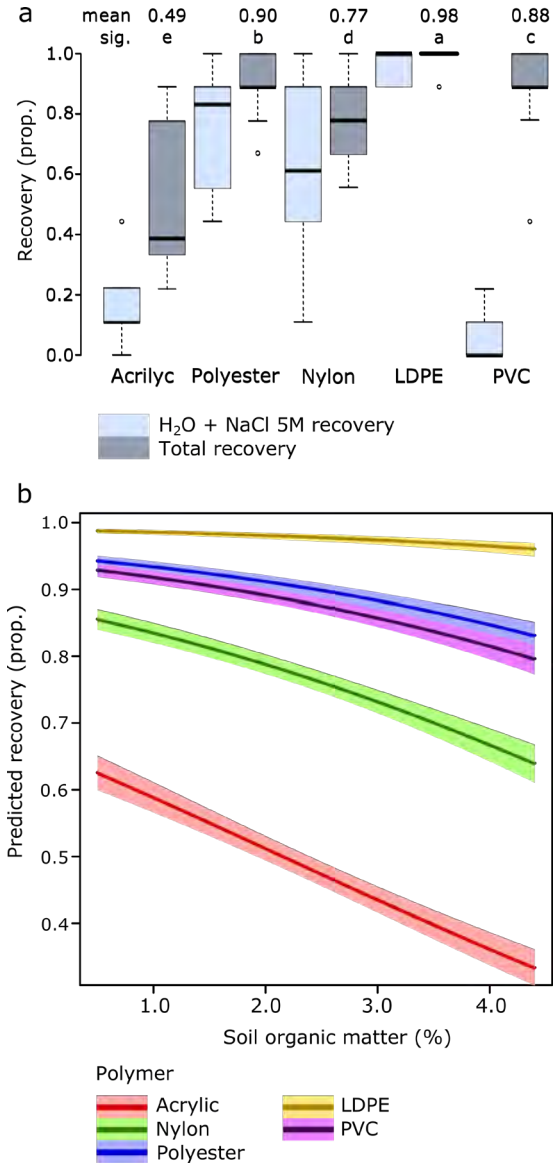

Figure 4.3 *Results of the validation test: (a) Recovery rates by polymer, splitting the recovery as total (grey) and by using only two steps (H_2O and NaCl 5M) (blue); (b) Predicted recovery rates by content of soil organic matter (CI = 95%).*

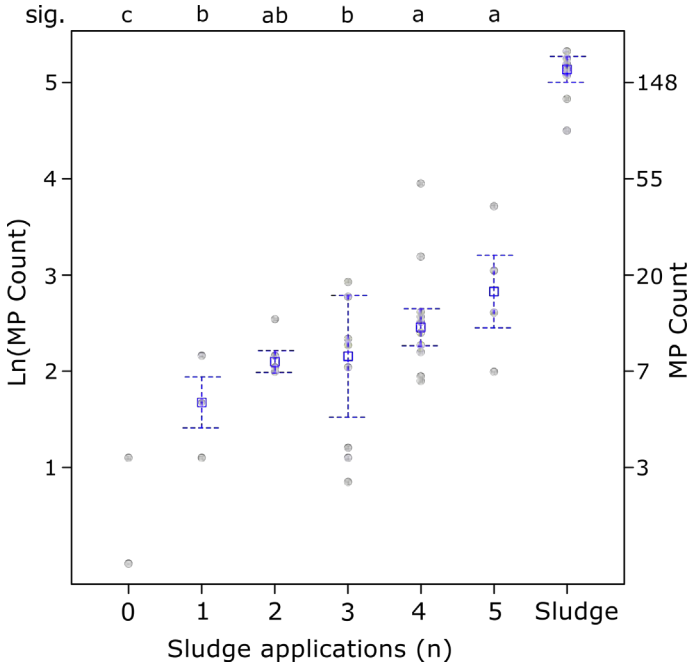

Figure 4.4 *Microplastic (MP) counts by number of sludge applications. Average of counts per field (grey dots), treatment medians (blue squares), and inter quantile range (blue dotted arrows). Differences at α <0.05 are shown in the top axis with lower case letters.*

Table 4.2 *Microplastic weight in soil by number of sludge application events and in sludge (mg kg^{-1}).*

Applications	Min	1st Qu.	Median	3rd Qu.	Max
1	0.73	1.05	1.37	1.78	2.18
2	1.79	1.90	2.03	2.38	3.16
3	0.57	0.79	2.22	2.97	4.56
4	1.76	2.25	2.88	3.38	12.9
5	1.79	3.03	4.38	6.56	10.3
Sludge	22.0	37.3	45.5	50.2	53.0

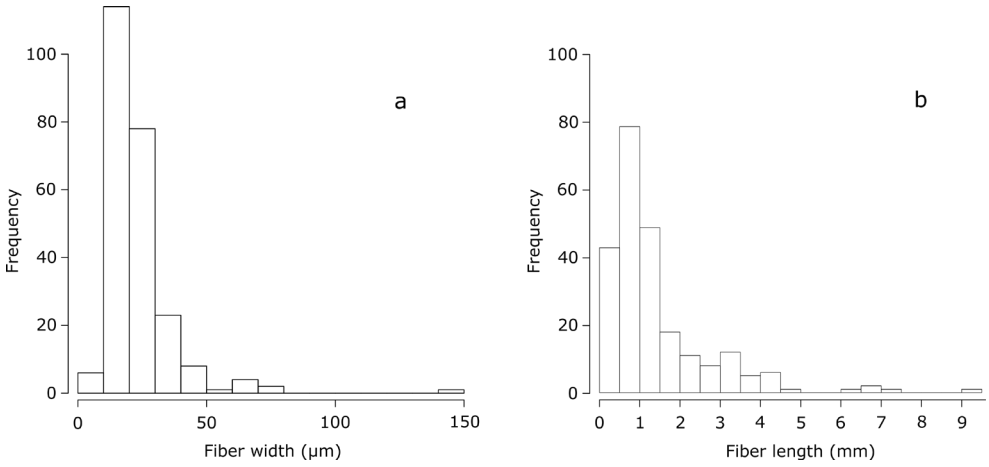

Figure 4.5 *Histograms for fiber width (a), and fiber length (b).*

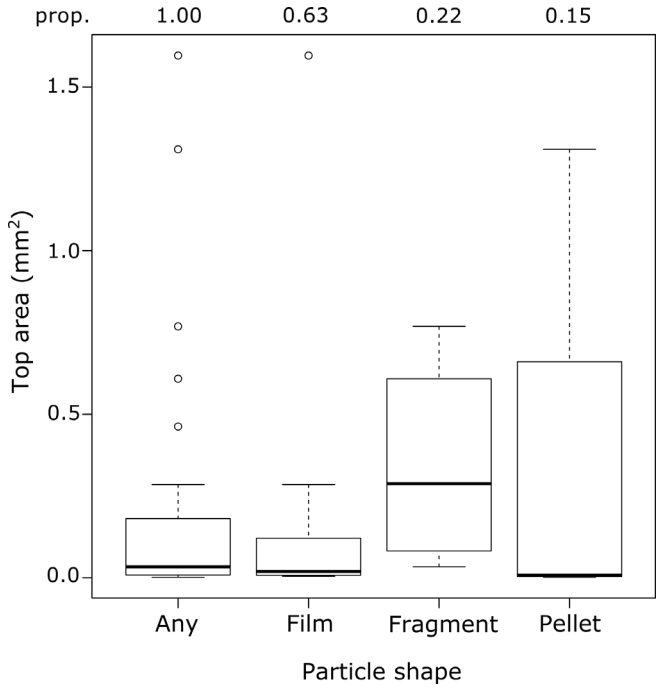

Figure 4.6 *Non-fiber particles surface area by shape.*

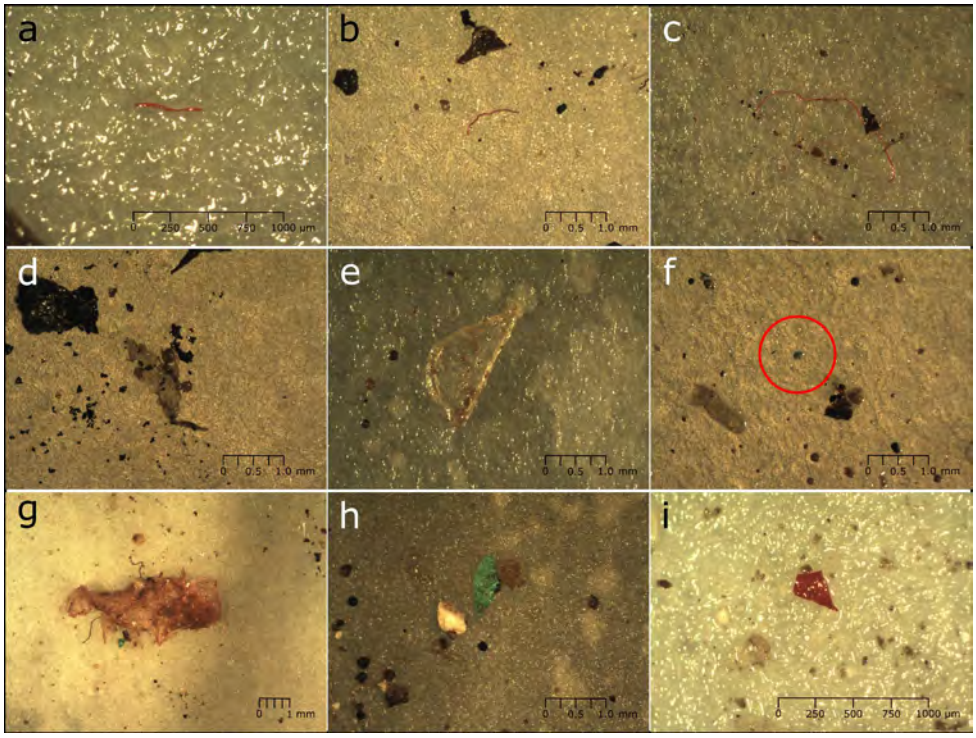

Figure 4.7 *Example images of the microplastics observed. Fibers (a, b, c), films (d, e), pellets (f), and fragments (g, h, i).*

Considering the fact that the ratio of fibers to total microplastic particles was different between sludge and soil samples, the question could be raised: Do non-fiber microplastic particles have a greater mobility in soil and thus, are they being washed out of fields while fibers remain? The movement of fiber-like particles through porous media involves complexities that differ greatly from other geometrical forms, as pointed out by Engdahl (2018). The author reported one of the first efforts in modeling microplastic movement in porous media. Although it was a simulated experiment, his findings indicate that sometimes counter-intuitive transport processes take place. The concept of microplastic mobility through the soil profile was reviewed in Rillig et al. (2017a) and in Steinmetz et al. (2016). Since our study presented limited data and had methodological restrictions, the microplastic content of the sludge samples was not directly comparable with the soil data. Hence, our initial question remains unanswered and is set as a challenge for future investigations. After all, if plastics are remobilized from soil to water bodies, the main aim of waste water treatment plants to prevent water contamination could be compromised by current sludge disposal methods.

4.4.2 Method limitations

We were able to quantify microplastic particles in sludge and soil samples as units per unit of weight with the method we used. Reporting results as units per weight has the advantage that simpler and cheaper methods can be used for soil evaluation. One benefit is that the method offers alternatives for microplastic detection for developing economies. For now, the method may be used in semiarid environments, with low organic matter and calcareous soils. The method was not fast so extensive assessment campaigns using this method could prove to be time consuming if used in other contexts. There are faster methods currently being developed but they have higher analytical costs (e.g. Corradini et al., 2019a; Paul et al., 2019). Reporting values as units per given weight is a known limitation since it could be difficult to compare results between studies (Bläsing and Amelung, 2018; Scheurer and Bigalke, 2018). So far, most studies assessing microplastics in soils present their data in this form (e.g. Huerta Lwanga et al., 2017b; Liu et al., 2018; Scheurer and Bigalke, 2018; Zhang and Liu, 2018; Zhang et al., 2018; Zubris and Richards, 2005). In some of these studies, researchers used diameter and average density to try and estimate the plastic content on a weight to weight (w/w) basis. The disadvantage of this is that uncertainty is increased because the converted data is only an approximation. For example, fibers had a void fraction which may vary and thus assumptions had to be made in order to perform conversions to w/w (Simon et al., 2018). Despite the uncertainty, new evidence shows that simultaneous quality-quantitative assessments are important for inference of effects (De Souza MacHado et al., 2018b).

Visual sorting depends on the operator's criteria. Therefore, additional identification techniques are often used to avoid false positives. These additional techniques were not included in our approach. Although it is advised to couple visual techniques with spectroscopic approaches, the use of such techniques considerably increases the cost and complexities of the analysis. The additional gains in accuracy may not be relevant depending on the study purpose. Horton et al. (2017) reported a 7% rate of misclassification (particles that were natural, as opposed to anthropogenic) when they validated the method (using soils with ~6% organic matter).

Organic matter removal steps were not included in the method used. These steps are used to avoid false positives during optical inspection. Commonly used reducing agents degrade microplastics. Hurley et al. (2018) reported that Fenton's reagent could be an alternative. However, when we tried the Fenton's reagent protocol with fibers, the recovery of nylon and acrylic fibers decreased considerably (data not shown). Organic matter removal is not always mandatory and may reduce extraction efficiencies (Wang et al., 2018). The removal of organic matter is recommended when organically rich samples with a high potential interference are analyzed (Fuller and Gautam, 2016; Mahon et al., 2017). The soils surveyed

had between 1.3% and 4.3% organic matter. This did not represent a problem during analysis.

Additionally, when the methodology was tuned up, we noticed that carbonates hindered organic matter removal (data not shown). For example, Fenton's reaction is partially impeded when carbonates are present (Liu et al., 2016). Therefore, if organic matter removal is needed, carbonates should be eliminated first. If this is the case, one should note that carbonate elimination is performed by acids (Soil Survey Staff, 2014), thus unnecessary particle abrasion could take place. The study sites were located in a semiarid region where carbonates range from 1.0% to 2.5% ($CaCO_3$). Consequently, organic matter removal steps were avoided, on the one hand due to the low organic matter, and on the other, due to the presence of $CaCO_3$.

Observed recovery rates were similar to other findings. Li et al. (2018) evaluated the recovery rate of polyethylene (LDPE) from sludge samples using NaCl extraction. These researchers reported a success rate of 86%, which is similar to the recovery observed in our experiements after the first two extractions. Similarly, using a higher density solution (NaI, ρ = 1.8 g cm-3), Hurley et al. (2018) recovered 92-98% of small LDPE beads, almost 100% of large LDPE beads, and 79-86% of polyester fibers. To our knowledge, there are no studies evaluating the recovery rate of nylon or acrylic fibers or PVC. High recovery rates may be due to the high density of 5M $ZnCl_2$ (ρ = 1.55 g cm^{-3}), compared to the densities of the polymers (ρ = 1.38, 1.17, and 1.14 g cm^{-3} for polyester, acrylic, and nylon (Qin, 2016), ρ = 1.35 g cm^{-3} for flexible PVC (Titow 2012), and ρ = 0.92 g cm^{-3} for LDPE (Zhang et al., 2018)).

There was evidence of a laboratory sample contamination. It could be considered negligible when compared to the observed microplastic counts. Zhang and Liu (2018) reported an average of ~4 fibers in control samples, which is comparable to the 2 fibers per filter paper reported by Horton et al. (2017). Exploring microplastic content in beach sand, Lots et al. (2017) reported an in lab contamination in 3 out of 5 control replicates (60%). Scheurer and Bigalke (2018) reported that 3 out of 9 blank filters were contaminated with fibers when evaluating Swiss floodplain soils, which is similar to the proportion observed in our work (30%). Sample contamination is a recurrent issue across current studies. To keep contamination at negligible levels, sample weight should be defined carefully. For the purposes of this study, using only 5g of a sample was sufficient to reduce the noise caused by sample contamination.

The repeatability reached by the method was good, being within the ranges expected for soil tests (McLain et al., 2018; Vaughan, 2018). The coefficient of variation (12%) implies that the probability of two replicate measurements differing by a factor of 1.5 or more is <0.025 (Reed et al., 2002). Repeatability has not been addressed directly in any of the

studies that propose new methodologies for soil microplastic assessment. Repeatability reflects how much of the variation between samples is due to the analytical method and how much is due to the treatments, thus it is an important indicator of quality control.

4.5 Conclusion

Sludge applications on soils resulted in increased microplastic counts in soil samples. By evaluating agricultural fields with different sludge application records, we provided evidence of microplastic accumulation over time. The data revealed a high concentration of microplastics in the soils, supporting the fact that sludge is a driver of soil microplastic contamination. Also, evidence showed that there could be plastic remobilization away from fields, revealing challenging new research questions.

The method can be used as a guide to examine microplastic contamination in semiarid regions but further methods need to be adapted for other environments. There are yet only a few studies addressing soil microplastic contamination, thus the true scale of the problem has yet to be assessed. Research on plastic weathering and transport processes within the soil profile are still pending and are greatly needed to understand the fate of the pollutants in the overall environment.

Chapter 5. Microplastics occurrence and frequency in soils under different land uses on a regional scale

The growing evidence of microplastic pollution in terrestrial ecosystems reveals adverse effects of microplastics on soil biota and plant growth. However, since large scale assessments are lacking, it is possible that the laboratory based experiments conducted have assumed unrealistic microplastic concentrations in soils. In this paper we present regional scale data on the presence of microplastics in soils under different land uses in the central valley of Chile, which is characterized by urbanization, agricultural, and mining operations. We identified microplastics in soils under four different land use systems having different management intensities (crop lands, pastures, rangelands, and natural grasslands), and all somewhat prone to accumulate microplastics from different sources. We analyzed 240 soil samples from Chile's central valley, trying to identify the most probable sources of the microplastics. Our hypothesis was that microplastics were ubiquitous in the environment and that their concentration peaks follow the intensity of fertilizer use (phosphorus), soil heavy metals concentrations derived from nearby mining operations (Zn and Cu), and distance to roads and urban areas. We did find evidence of microplastic pollution in crop lands and pastures (306 ±360 and 184 ±266 particles kg-1, respectively), but we did not observe pollution of rangelands and natural grasslands. Distance to mining operations, roads, or urban areas did not increase the microplastic particles count. Our observations contradict the common belief that microplastics are ubiquitous in the environment and relates the pollution problem more to agricultural activities. However, our data do not provide sufficient evidence to identify the pollution source. This is the first study that reports on microplastic occurrence in soils at a broad geographical scale. For greater insight on this topic more studies that contribute monitoring data about microplastics in soils are urgently needed.

Based on:

Corradini, F, F Casado, V Leiva, E Huerta-Lwanga, V Geissen. 2021. Microplastics occurrence and frequency in soils under different land uses on a regional scale. Science of the Total Environment 752:141917.

5.1 Introduction

In recent years the scientific community has recognized plastic pollution as a major environmental threat (Isobe et al., 2019). Their concerns eventually reached society (Henderson and Green, 2020) and, as a consequence, policy-makers began drafting the first regulations to mitigate the problem at its source (e.g. EU Directive 2019/904). In the first years, pollution of aquatic ecosystems by microplastics (particles less than 5 mm in diameter) monopolized the debate, since scientists fist observed these pollutants in marine ecosystems (Carpenter and Smith Jr, 1972). After four decades, soil scientists revealed a new angle to the problem by exposing the presence of microplastics in terrestrial environments (Rillig, 2012). Today, soil scientists are still working to better understand the problem and its ramifications, primarily focusing on determining the magnitude of the problem (i.e. frequency of occurrence and potential effects). In their work, they stress the need for more field data on the occurrence of microplastics.

To date, soil scientists have gathered evidence that suggests the major pathways by which microplastics are introduced into soils. The evidence indicates that the application of soil amendments such as compost (Cattle et al., 2020; Watteau et al., 2018; Zhou et al., 2020a) and sludge (Corradini et al., 2019c; Edo et al., 2020; van den Berg et al., 2020; Zhang et al., 2020b) transfer and disperse microplastics from sinks of urban wastes to agricultural lands. Plastic mulches used in agriculture constitute another major source of pollution (Ding et al., 2020; Huang et al., 2020a). Other pollution sources have received less attention. For example, few studies explore the atmospheric deposition of microplastics in soils (Zhang et al., 2019), although the presence of microplastics in all atmospheric compartments has been documented (Mbachu et al., 2020).

The efforts made to identify the pathways by which microplastics are introduced to soils lag behind the efforts made to assess the frequency of occurrence of such pollution. Most studies have focused on the identification of specific pollution sources under one land use condition (agriculture), and seldom report large scale assessments. Scheurer and Bigalke (2018) published the only study to date that assessed microplastic pollution at a regional level. The authors sampled floodplain soils in Switzerland and quantified the microplastics pollution. Although their study shed light on the ubiquity of microplastics in terrestrial ecosystems, scientists have seldom tested the hypothesis in other —different— environments (see Zhang et al. (2019) for one of the few examples).

Along with the evidence gathered on microplastic sources and fate in terrestrial ecosystems, soil scientists have reported the effects that microplastics soil pollution has on crops, soil biota, and the trophic chain. These studies have primarily taken place under laboratory conditions where researchers use incubation techniques to measure

toxicological effects of microplastics alone or combined with other pollutants. As a result, scientific journals have begun to amass evidence on the hyperaccumulation of agrochemicals (Ramos et al., 2015) and heavy metals (Yu et al., 2020; Zhou et al., 2019) on microplastics' surfaces, and the adverse effects of microplastics on soil biota growth and development (Huerta Lwanga et al., 2016; Jiang et al., 2020; Qi et al., 2018; Selonen et al., 2020; Zhou et al., 2020b). However, as Piehl et al. (2018) acutely strongly argued, laboratory-based toxicological studies can be criticized as their methods presuppose concentrations that may be unrealistic. The authors contend that studies and evidence of soil microplastic pollution needs to cover different ecosystems and land uses to overcome this limitation.

Based on the research done to date, the presence of microplastics across different land uses remains unknown. This unknown jeopardizes the chances of more ambitious research questions dealing with toxicology and/or mitigation on larger scale. We set out to address this knowledge gap. Therefore, we conducted a regional scale study on the presence of microplastics in the topsoil under a variety of land uses with different management activities in Chile's central valley. In addition, using the new data, we evaluated the possible concurrence between microplastics pollution and intensive agricultural practices, dry/wet deposition as a result of offsite transport from roads/urban areas, and aeolian transport from mining activities.

5.2 Materials and methods

5.2.1 Study area

5.2.1.1 General description

The soil samples analysed in this research were collected from Chile's Región Metropolitana, an area of 1,539,658 ha situated at the north end of Chile's central valley (Fig 5.1). The samples were collected during a previous soil monitoring effort (2017) and were archived to be available for other study purposes. The study area comprised soils under one of the following land uses: arable soils (crop lands) used for agriculture (228,284 ha), pastures (19,523 ha), rangelands dominated by shrubs (244,817 ha), or natural (native) grasslands (22,735 ha) (CONAF and CIREN, 2013). Chile's Región Metropolitana lacks a comprehensive soil survey. However, the government provides information about soils used or with potential use for agriculture, which correspond with our study area. According to those soil maps, Mollisols predominate (70%), followed by Alfisols (11%), Inceptisols (13%), Entisols (2%), and Vertisols (4%) (CIREN, 1996).

The Köpen-Geiger climate classification map indicates a 'warm temperate' climate with warm summers and low precipitation (map unit = Csb) (Kottek et al., 2006). Mean annual precipitation is about 300 mm, and the average annual temperature is 15 °C, with 23 °C maxima and 10 °C minima. Due to the climate, the soils within the study area present a xeric moisture regime and a thermic temperature regime (CIREN, 1996). The predominant wind direction changes throughout the year together with changes in circulation and precipitation. Northerly winds predominate during autumn and winter —the rainy season. The Pacific High causes shifts in the wind direction to South-West during the spring and summer (Olivares et al., 2002).

Three topographical features characterize the region. The Andean range rises to the East, while another mountain range defines the landscape to the West; the Chilean coastal cordillera. Between these mountain ranges two major basins compose a valley at 600 m amsl. This valley is disconnected from what is toit's the North and South, since the coastal cordillera intrudes towards the valley reaching the Andes at the region's boundaries. Two major rivers drain the valley. The Maipo River ($92.3 m^3 s^{-1}$), flowing East-West, drains the valley's southern part. The Mapocho River ($6.1 m^3 s^{-1}$), flowing North-South, drains the northern side of the valley reaching the Maipo River as a right-bank tributary.

Santiago —Chile's largest city— lays in the central-East part of the valley. It is home to 5.6 million inhabitants. Since the city has a relevant role in the country economy, there is an extensive road network in its surroundings totaling 2,296 km of asphalt (Albers and Albers, 2019). Mining is Chile's primarily economic activity. Within Chile's Región Metropolitana, there are nine ongoing major mining operations. Three of them exploit ore veins in the Andean range, while the other six do so in the costal cordillera (Albers and Albers, 2019).

5.2.1.2 Possible pathways for microplastics introduction to soils in the study area

We considered four potential sources of microplastics pollution. Agriculture, mining, roadways and the urban environment.

We suspected that agricultural activities might increase microplastic counts. There is evidence that supports this regarding the use of plastic mulch or sewage sludge as fertilizers (Piehl et al., 2018; Zhou et al., 2020a). In the study area —according to the records of the Chilean Agricultural and Livestock Service— none of the sites from which the samples came were used for sludge disposal or had cropping systems that involved the use of plastic mulches or plastic covers. Although government information on land use stands as an indicator of agricultural activities, it does not say anything about their intensity. We used soil P concentrations as a gauge of anthropic pressure, since previous scientific reports have reported that the area undergoes overfertilization practices with phosphate fertilizers

(Corradini et al., 2019b). In other words, we expected the highest microplastic counts in crop lands, and that the counts would increase together with soil P.

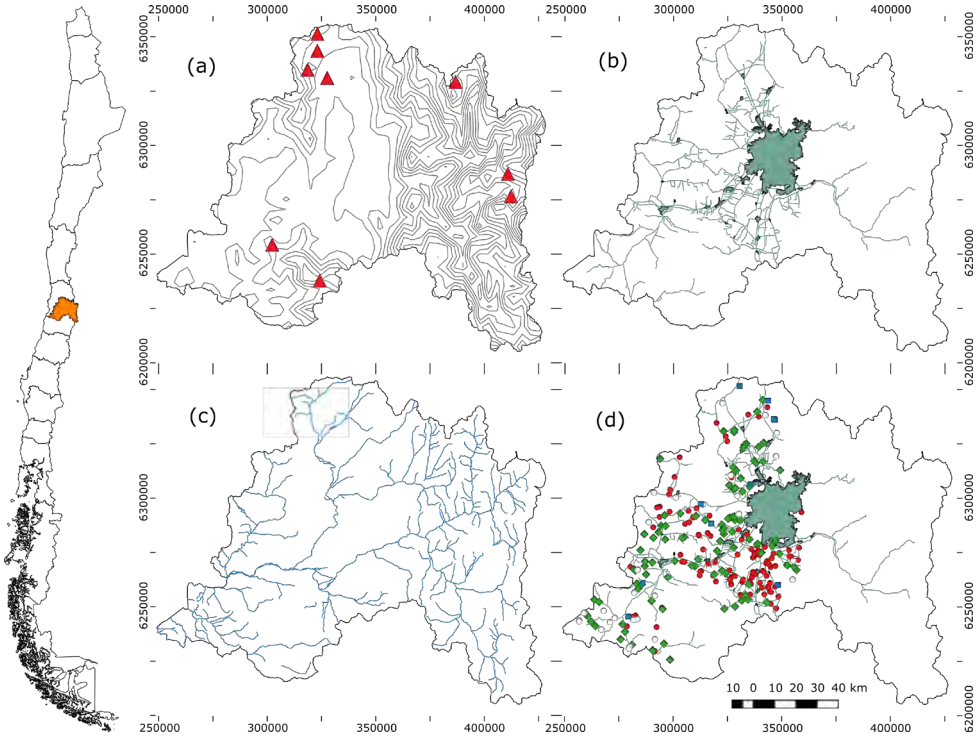

Figure 5.1. *Study area. Left: general location map with the country borders. Chile's Región Metropolitana is highlighted in orange. Top-left (a): Región Metropolitana with contour lines every 250m. Red triangles mark the place of ongoing mining operations. Top-right (b): Región Metropolitana and its asphalted roads, and urban areas. Bottom-left (c): River networks of Región Metropolitana. Bottom-right (d): sampling points by land use. Red circles mark crop land, green diamonds mark pastures, white circles mark grasslands dominated by shrubs, and blue squares mark natural grasslands. Coordinates are WGS 84 / UTM zone 19S in kilometers.*

Also regarding agriculture, we consider compost as a potential introduction pathway of microplastics to soils. It has been reported that compost can transport microplastics to soils (Watteau et al., 2018). Although farmers did not use sewage sludge as an organic amendment in the crop lands within our study area, some of them may have used compost. In the study we used archived samples (see section 5.2.2), and unfortunately the samples' accompanying information did not report management information, such as compost use. However, compost is sometimes used by farmers in the area and scientific studies indicate

that compost increases soils' EDTA and total Zn and Cu after repetitive applications (Cambier et al., 2019). Therefore, we expected higher microplastic counts in soils that had relatively higher Zn or Cu than other soils of the region.

Besides the effect of agriculture, we considered mining as a potential source of microplastics pollution. Plastic pipes are used during mineral extraction for cooling and ventilating processes. It has been reported that plastic pipes may release plastics to the environment as they abrade during their lifetime (Sargand et al., 2013). Therefore, mining could be a source of microplastics as wind might carry microplastics from decaying pipes — or other industrial operations— to offsite soils. Since mining activities enrich nearby soils with Cu by aeolian processes (Neaman et al., 2020), we expected microplastic counts to increase wherever soil Cu concentrations increased.

The other two potential introduction pathways were also related with aeolian deposition of microplastics. On the one hand, based on the argument of Bläsing and Amelung (2018), we considered roads. We expected microplastic counts to increase as distance to roads decreased. On the other hand, we considered the city of Santiago as a potential source of microplastics. Researchers have recognized the city's role as a pollution source for nearby ecosystems (Cereceda-Balic et al., 2012). Therefore, we expected microplastic counts to increase as the location of the soil samples approached urban area.

5.2.2 Archived soil samples

All the soil samples analyzed for this study came from a previous soil monitoring effort conducted by the Chilean Agricultural and Livestock Service (Corradini et al., 2019b). The service took 480 samples distributed across different land uses and soil types within Chile's Región Metropolitana for monitoring purposes and archived the samples in 2017. Sample locations were defined by a conditional Latin hypercube algorithm. The algorithm stratifies the sampling according to exhaustive ancillary data and provides full coverage of the range of each variable, assuring representativeness of the underlying information (Minasny and McBratney, 2006). Corradini et al. (2019b) report all sampling campaign details. Briefly, they used land use, land cover, and soil survey maps together with topography to place the sampling points in representative places within the basin. They collected a total of 480 samples from which, for this study, we randomly selected half of the samples for every land use considered. We analyzed 100 samples from crop lands, 100 from pastures, 30 from rangelands dominated by shrubs, and 10 from natural grasslands.

5.2.3 Soil analysis

Since the soil samples came from a previous monitoring effort, they were ready for analysis (i.e. they were dried and sieved < 2 mm). The Chilean Agricultural and Livestock Service had measured available phosphorus (P) by the Olsen method, thus, to test our study hypothesis we needed to analyze in addition total zinc (Zn), and total copper (Cu). To measure Zn and Cu, 2 grams of soil per sample were acid-digested (EPA Method 3050B, EPA (1996)) and analyzed by flame atomic absorption.

5.2.4 Microplastics analysis

We used two different methods to detect microplastics in the soil samples. Both have a similar extraction procedure (section 5.2.4.1), but use different instruments to detect microplastics, and have different purposes. On the one hand, we used a visual identification and sorting method to count microplastic particles (section 5.2.4.2). On the other hand, we reanalyzed the samples in which we found microplastics (n = 93) with an FTIR microscope to identify the frequency of different polymer types (section 5.2.4.3).

5.2.4.1 Extraction

The extraction steps followed to prepare the samples for the visual identification method were the same as reported for a previous study (Corradini et al., 2019c). Briefly, glass centrifuge tubes holding 5g of soil and 20ml of water (1.00g cm^{-3}) were centrifuged (15min at 2,000rpm) and the supernatant filtered through Whatman No.42 filter paper. After the supernatant recovery, the tubes holding the remaining sediments were filled with 20ml of sodium chloride (5 M ρ = 1.20g cm^{-3}), stirred (30s at 21,000rpm) and centrifuged to filter the supernatant a second time. The tubes with the sediments were filled a third time with 20ml of zinc chloride (5 M, ρ = 1.55g cm^{-3}), stirred and centrifuged one last time. The supernatant was then filtered through the same filter used the previous two times. After the extraction, the filters were saved in Petri dishes for optical inspection.

To prepare the samples for the FTIR microscope, we used a method also based on density separation. Here, the glass tubes held 1g of soil and 10ml of zinc chloride ($ZnCl_2$ 5 M, ρ = 1.55 g cm^{-3}). The tubes were placed in an ultrasonic bath for 10 minutes and then agitated in a vortex shaking machine at 2,000rpm for 15s. Later, the tubes were shaken in an orbital shaker for 20min at 180 oscillations per minute. Before filtration, the tubes were centrifuged 10min at 2,500rpm. The supernatant was vacuum filtered through Whatman™ Cyclopore™ Polycarbonate Membrane Filters (diameter = 25mm, pore size = 0.4µm). The tubes were re-filled with zinc chloride and underwent the same steps described once again. After filtration, the polycarbonate membrane filters were rinsed with distilled water to

transfer the captured particles to Whatman® Anodisc inorganic filter membranes (diameter = 25mm, pore size = 0.4µm, with a polypropylene support ring). This step was needed since the polycarbonate membranes cannot be used with the FTIR microscope due to their low transparency. The Whatman® Anodisc membranes were dried at 40°C for 12h before the FTIR inspection.

5.2.4.2 Identification – optical microscope

After the extraction process, the filters were inspected using a stereo microscope (model SMZ 745 T coupled with a NI-150 high intensity illuminator, Nikon, Tokyo, Japan) at 20x. All microplastic particles collected on each filter were counted twice. Objects with shiny surfaces, strong colors, and sharp geometrical shapes were considered to be microplastic particles. Objects with smooth sides and strong colors, were considered to be synthetic fibers, as described by Horton et al. (2017). Particles were classified according to their shapes as: fibers, fragments (angular and solid), films (flexible and thin), or pellets (rounded and solid). Every microplastic particle observed was photographed (Micrometrics® camera model 519CU CMOS 5.0 Megapixel, ACCU-SCOPE Inc., NY, USA) to measure the length if fibers and the surface area if film, fragment, or pellet. ImageJ 1.5 software was used for this purpose (Schneider et al., 2012). Results were reported as number of microplastic particles per g of dry soil.

5.2.4.3 Identification – FTIR microscope

After the extraction process, the filter membranes were scanned in an Agilent µFTIR Microscope and Bench that combines both a microscope (Cary 620) and the analytical bench (Cary 670) capabilities for Fourier Transform Infrared Spectroscopy (FTIR) (Agilent Technologies, Inc. CA, USA). The equipment records the infrared absorbance spectra of all the particles that lay on top of a target membrane. It does so by registering the transmittance of a laser beam that goes through —step by step— all the particles that lay on top of the membrane at each of the coordinates of the microscope tray. The spectra is recorded in a grid of pixels, each pixel representing a given coordinate on the microscope tray. The area that each pixel represents depends on the objective lens placed in the microscope and is reported as the length of the pixel side.

The images captured by a FTIR microscope can be used to identify (and count) microplastics by contrasting the spectra of a particle with the reference spectra of a known polymer. Reference libraries that compile spectral signals for different polymers are available for microplastic analysis (Primpke et al., 2018). Bulk search algorithms, and other computational methods, use these libraries to automatize the process of identifying plastic polymers (Primpke et al., 2019a; Wander et al., 2020). The soil samples were analyzed in transmission mode with a spectral resolution of $8cm^{-1}$ through a spectral range from 3500

to 1300cm^{-1} and 8 co-added scans. Data was recorded in absorbance (%). The microscope magnification was x4 for a pixel size resolution of 20.6µm.

We used an automated image classification approach to identify microplastics among the buoyant particles that were trapped on the membranes and registered in the µFTIR images. The approach consisted of a library search routine based on the spectral angle mapper algorithm. It included image pre-processing, spectral matching, and image post-processing. The image pre-processing involved three steps; scaling the spectra, calculating its first derivative using a Savitzky-Golay filter, and resampling the spectra to cut out the CO_2 peak. The spectral matching, or image classification, was done using the spectral angle mapper algorithm. The algorithm finds the best match for each pixel by contrasting the pixels' spectra with all polymers available in the reference library. It summarizes the likeness as an angular degree; the smaller the degree, the greater the likeness. The algorithm recognizes different polymers successfully (Wu et al., 2020b), and to this end the waste recycling industry has used it for over 15 years (Kulcke et al., 2003). The post-processing included clipping the image to remove the membrane's polypropylene support ring, filtering all pixels that did not have a good match with any of the reference polymers (angular degree >= 1.2°), and smoothing the image using a 3x3 moving window (i.e. kernel convolution). The process output is the number of particles classified by polymer type and their area. Following this procedure, the limit of detection is 1273µm^2. The complete process is implemented in the R package 'uFTIR' (Corradini, 2020), which works within the R environment for statistical computing (R Core Team, 2019).

5.2.5 Quality control

5.2.5.1 Visual identification of microplastics

Each set of samples (n = 20) included one reagent blank. The filter corresponding to it was saved in a Petri dish and inspected at the end of the analysis. This way, the blank accounted for both the quality of the reagents used and contamination inside the lab (Mahon et al., 2017; Scheurer and Bigalke, 2018). All materials used in the analysis were made of glass (funnels, Petri dishes, centrifuge tubes) and the stirrer rod was made of stainless steel. White cotton lab coats were used by analysts during analysis and sample manipulation. We observed plastic particles in 5 out of 15 blanks. One count per each of the 5 polluted blanks. All of them were fibers (mean length = 1µm).

Regarding the method's repeatability, the standard deviation of the mean ranged between 0.05 and 0.10 particles per gram. The expected difference between replicates (standard deviation) from 0.10 to 0.20 particles per gram.

As previously described, the samples came from a previous monitoring effort and were stored in PET flasks for two years before the analysis. This circumstance hampers microplastics analysis since in lab contamination might occur and yield false positives. However, we only observed one polyester particle in the µFTIR analysis (Section 5.3.2) and fibers were the most commonly found plastic shape (Section 5.3.1). Both results suggest a low degree of sample contamination due to sample storage.

5.2.5.2 FTIR microscope –image acquisition

Each set of samples (n=28) included two blanks. All of them (n=8) had 0 particle counts except one that had one plastic particle of rubber (area = 1360µm^2). However, no rubber particles were found in any of the 93 soil samples scanned. Therefore, we considered cross contamination for this method negligible.

5.2.5.3 FTIR microscope –validation test for image processing and polymer recognition

The spectral angle mapper algorithm discriminates well between polymers, and both scientists and industry have used it to classify plastic polymers (Wu et al., 2020b). However, we tested whether it was correctly implemented in the 'uFTIR' package. To do so, we recorded the spectra of one polyethylene bag, two plastic cups —one made of polypropylene and the other made of polystyrene— and a polystyrene standard film (VARIAN P/N 883-9120). A single image of 128x128 pixels was recorded and analyzed for each polymer with the same settings we used to acquire the image of our samples. We expected the program to match the polymer we placed in the microscope tray for all the pixels in the image. This was the case for all polymers with a tolerance of 4% (Table 5.1). The package 'uFTIR' correctly classified all pixels of the standard polystyrene film, and almost all pixels of the polystyrene cup. The algorithm was confused in 1% of the cases when it classified the polypropylene cup, wrongly attributing 88 pixels to polyethylene (i.e. 1% of the total). The analysis of the polyethylene bag had the lowest success rate, misclassifying 4% of the pixels. However, the algorithm attributed those pixels to ethylene-vinyl-acetate, which is a polymer composed by polyethylene and vinyl-acetate in a ratio from 10:1 to 10:4.

Table 5.1. *Polymers scanned and analyzed in the validation test: number of particles detected (Part.), total area and proportion of the total area (prop.), other polymers identified in the same image, and the area of those other polymers.*

Polymer	Part.	Area		Other	Area
	n	pixel^2	prop.	—	pixel^2
polyethylene	2	15,705	0.96	ethylene-vinyl-acetate	679
polypropylene	1	16,296	0.99	polyethylene	88
polystyrene	1	16,351	>0.99	polypropylene	33
polystyrene standard	1	16,384	1	—	0

5.2.6 Statistical analysis

To test whether microplastics were ubiquitous in the study area, we checked whether microplastic pollution could be expected in all land uses and whether or not thepollution chances were equal across them. We used descriptive statistics —median and interquartile range— to check whether all land uses had at least one sample polluted with microplastics. We used a logistic model to check whether the chance of pollution was equal across land uses. The logistic model regressed microplastic pollution on land use. Sample distance to roads and urban areas were used as a covariable. Microplastic pollution was considered a dichotomous variable equal to 0 when no microplastics were observed in a sample, or 1 otherwise. Land use was a categorical variable with four levels reflecting each land use. Distance to roads and urban areas were continuous variables expressed in meters. In its output, the logistic model tells whether land use affects the chances of finding microplastics in a sample, and how much the chance increases or decreases compared to a base or reference class (land use). The reference class we defined for the analysis was rangelands dominated by shrubs. Regarding samples' distance to roads and urban areas, the analysis tells whether the distance to them affects the chance of finding microplastics, and how much these chances increase or decrease as distance increases by one unit. Significance was evaluated at p-value <0.05.

We did a Kruskal-Wallis test to look for differences in microplastic counts between land uses. The goal of the test was to test the significance of the observed differences in the counts, given that a sample was polluted. Therefore, only samples that had a positive —non zero— microplastics count were considered for the tests. In other words, the Kruskal-Wallis

test evaluated differences in microplastic counts between crop lands and pastures when we observed counts greater than 0. Natural grasslands and rangelands dominated by shrubs where not considered in the Kruskal-Wallis test, as they had only a few observations with counts greater than 0.

To check whether the concentration peaks of microplastics concur with the overaccumulation of fertilizers (P) or human-related heavy metals (Zn, Cu), we did a correlation test. Microplastic counts (visual method) were correlated with P, Zn and Cu concentrations (Spearman correlation). Asymptotic p-values determined the correlation significance (<0.05).

5.3 Results

5.3.1 Frequency of occurrence and expected quantities

Less than half of the samples analyzed contained microplastics (43%). The occurrence of microplastics varied by land use (Fig 5.2). Microplastics polluted more than half the samples from crop lands (57%), and a little less than half of the samples from pastures (44%). Microplastic pollution was less frequent in natural grasslands (20%) and rangelands dominated by shrubs (3%) (Table 5.2).

Table 5.2. *Microplastic counts by land use (visual method). Total samples by land use (n), number of samples that had a positive plastic count (p), and total counts of each plastic shape by land use.*

Land use	n	p	Film	Fiber	Fragment	Pellet
Croplands	**100**	**57**	**39**	**101**	**6**	**6**
Pastures	**100**	**44**	**18**	**63**	**10**	**0**
Rangelands	**30**	**1**	**0**	**1**	**0**	**0**
Natural grasslands	**10**	**2**	**0**	**2**	**0**	**0**
Total	**240**	**104**	**57**	**167**	**16**	**6**

The logistic model also showed that land use affects the chances of finding microplastics in a soil sample. Samples from crop lands and pastures had, respectively, a 38% and 23% higher chance of presenting microplastic pollution than samples from rangelands dominated by shrubs and natural grasslands. Neither distance to urban areas nor distance to roads influenced the chances of finding microplastics in the soil samples.

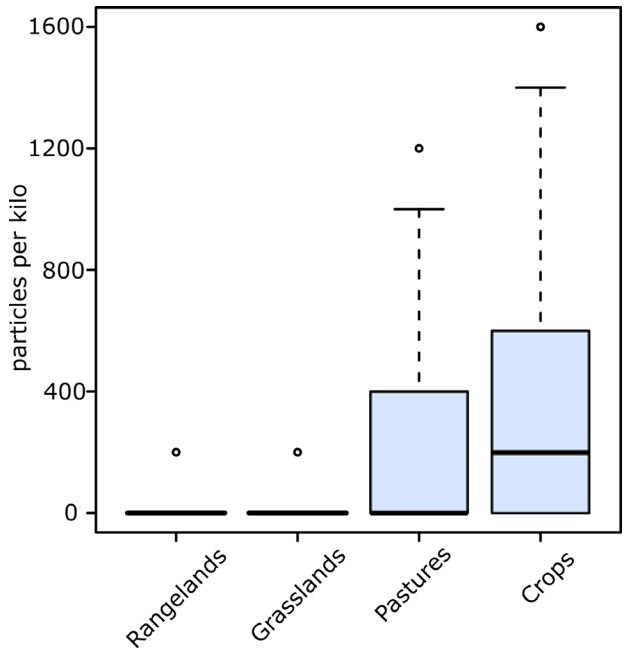

Figure 5.2. *Boxplot showing the median, interquartile range, minimum, and maximum counts of microplastics observed by land use.*

The Kruskall-Wallis test indicated that, among the samples that were polluted with microplastics, those from crop lands had higher microplastic counts (540 ±320 particles per kilo) than those that belonged to pastures (420 ±240 particles per kilo). The small proportion of samples that had positive microplastic counts and belonged either to rangelands dominated by shrubs (n = 1) or natural grasslands (n = 2) had only one microplastic per filter each (i.e. <=200 particles per kilo).

Across all land uses, fiber was the most common microplastic shape (68%), followed by films (23%). Fragments and pellets were observed less frequently (7% and 2%) (Table 5.2). The median area for particles other than fibers was 0.20mm^2, and the median length for fibers was 1.6 mm. The smallest area observed was 0.005mm^2, and the shortest fiber was 0.3mm. Figure 5.3 shows the size distribution for fibers and non-fiber shapes.

Median concentration of soil available P was 42mg kg^{-1}, with an interquartile range (IQR) equal to 52 mg kg^{-1}. Total Zn and Cu concentrations showed a median of 150mg kg^{-1} and 109mg kg^{-1}, and an IQR of 119mg kg^{-1} and 39mg kg^{-1}, respectively. No correlation was observed between microplastic counts and soil available P total Zn, or total Cu. Table 5.3 shows the concentration by land use.

Table 5.3. *Concentration of P-Olsen, Zn, and Cu by land use (mg kg^{-1}).*

Land use	Analysis	Median	IQR
		mg kg^{-1}	
Croplands	P-Olsen	42	38
	total Zn	135	117
	total Cu	58	32
Pastures	P-Olsen	43	7
	total Zn	159	121
	total Cu	57	44
Rangelands	P-Olsen	34	39
	total Zn	138	116
	total Cu	42	40
Natural grasslands	P-Olsen	50	39
	total Zn	144	83
	total Cu	57	15

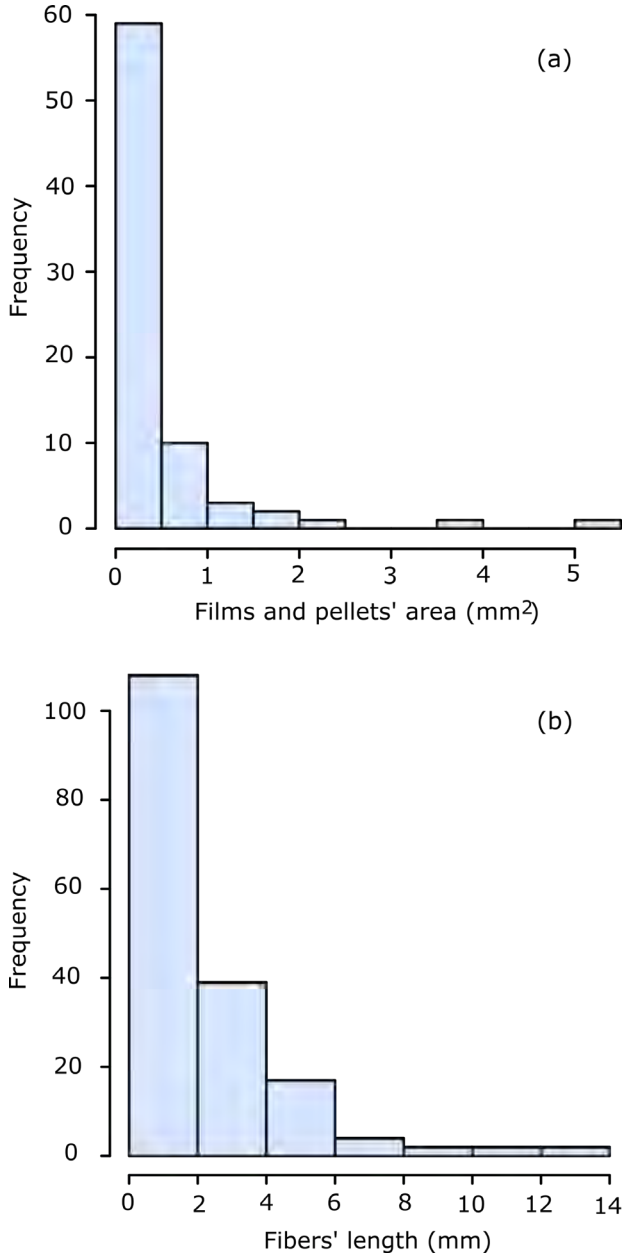

Figure 5.3. *Histograms for particle's area (films and pellets) (a), and fibers' length (b). Measurements done with the optical microscope and ImageJ for all microplastics identified by the optical —visual—method despite the land use to which they belonged.*

5.3.2 Commonly found plastic polymers

The µFTIR analysis detected microplastics in a few samples from the 93 samples we scanned. It showed that acrylates, polyurethane, and varnish were the most common plastic polymers found in the study area (Table 5.4). They were primarily observed in samples that belonged to crop lands. Polyethylene was the second most common polymer type. It was followed by polypropylene, nitrile rubber, and polystyrene. Table 5.4 shows the comprehensive list of plastic polymers identified.

Particles observed with the FTIR microscope had an average area of 0.076 ±0.292mm^2, with a median area of 0.012mm^2. The largest particle area was 2.036 mm^2. Particles this large were the exception; the second largest particle had an area one tenth this size (0.290mm^2). If the largest particle is not considered in calculating the average, the area becomes 0.035 ±0.058mm^2 (an area equivalent to the area of a circle of diameter 150 ±190µm^2).

Table 5.4. *Total observations by plastic polymer and land use.*

Polymer type	Crops	Pastures
Acrylates, polyurethane, and varnish	**10**	**2**
Polyethylene, and ethylene vinyl acetate	**8**	**3**
Polypropylene	**4**	**2**
Nitrile rubber	**4**	**1**
Polystyrene	**4**	**0**
Polyethylene chlorinated	**2**	**1**
Polyester	**1**	**1**
Polyamide	**1**	**0**
Polylactide acid	**1**	**0**

5.4 Discussion

In this regional scale study evaluating the presence of microplastic particles in soils under different land uses, we found clear evidence of microplastic pollution in crop lands and pastures, but not in rangelands or natural grasslands. This indicates that, while human interventions increased microplastics accumulation in soils in the study are, microplastics is not a widespread problem across all land uses. Despite the evidence of microplastic pollution in managed soils, we could not identify a possible pollution source nor a covariable to measure along with microplastics. Microplastic concentrations and occurrence were not correlated to high available phosphorus, nor total Zn or Cu. Along the same lines, distance to urban areas and roads did not affect the chances of finding a sample polluted with microplastics. In other words, our data suggest that microplastics pollution of soils happens in crop lands, regardless of fertilizers use, or compost applications; And that mining operations, roads and nearby urban areas do not contribute significantly to microplastics soil pollution in this region.

To date, there are no studies that report microplastic pollution on a regional scale across different land uses. Scientists have focused mainly on particular land uses or management scenarios. Our study offers the first insights into how widespread microplastics are in terrestrial environments where soils have not received direct inputs of plastics via sludge applications or plastic mulches. Our study also suggests that the chance of microplastics pollution is higher in managed lands compared to unmanaged lands. This contrasts with the findings of another rather large scale study on microplastics in soils. Scheurer and Bigalke (2018) sampled swiss floodplain soils and observed microplastics in almost 90% of the samples. Although the soils were unmanaged, river floods deposited plastic debris on the soil. The authors found microplastics also in floodplains with no human settlements upstream. They speculated that in those areas aeolian deposition acted as the transport agent since they observed only small microplastics there (<500µm). Our observations did not follow these findings. We did observe microplastics as small as 300µm for fibers and 80µm for other shapes in unmanaged lands. However, the frequency of occurrence was considerably lower. We found microplastics in only one sample that belonged to rangelands and two that belonged to natural grasslands and the count of microplastics for all three samples was the same: one fiber. In fact, contrasting the data with our blanks, it is conceivable that those fibers came from in-lab pollution and not from the soil samples themselves.

Our results can be better compared with what Piehl et al. (2018) observed for a German farm. These authors published the only study so far that addresses pollution in crop lands without direct (known) plastic inputs by way of sludge disposal and the use of plastic

mulches. They observed microplastics pollution in soil samples that belonged to a 0.5 ha farm in Germany. As in our case, they found microplastics in the farm soil, despite there being no clear pollution source. They reported a maximum of 1.25 particles per kilogram of soil; a number several times lower than what we observed (540 ±320 particles per kilo). That said, their analytical methods were different from ours. They looked only for microplastics over 1 mm in length, and therefore found fibers only over 2 mm in length. Our observations suggest that such long fibers are the least common, occurring in 35% of cases in our study (Fig 5.3). Also, the authors sampled a larger volume of soil than we did, which likely resulted in a considerably lower detection threshold. Using our method, Piehl et al. (2018) might have misclassified their farmland as a false negative. In this regard, the frequency of occurrence we report in section 5.3.1 is rather optimistic.

There are very few studies in the scientific literature that evaluate the concurrence of microplastics and other indicators of anthropic pressure. This gap is unexpected since the first study that reported microplastics in soils correlated the occurrence of synthetic fibers with soil Zn and Cu (Zubris and Richards, 2005). Contrary to what we observed, the authors reported a high correlation with both elements in their study area. However, that study site was tied to soils under regular sludge applications, which have the effect of increasing soil Zn and Cu. Despite this evidence, other studies that relate microplastic pollution with sludge applications have not addressed the concurrence of microplastics and Zn, or Cu, or any other pollutant (Corradini et al., 2019c; van den Berg et al., 2020; Zhang et al., 2020b). Beyond sludge applications, (Zhou et al., 2019) published the only study to date that evaluates the correlation between heavy metals and microplastic pollution. The authors sampled soils under three different land uses and observed that the higher the concentration of heavy metals the higher the microplastic count. Their findings counter ours. However, their study area comprised only peri-urban soils, where high heavy metal contents were expected, and thus it is not directly comparable to our study location.

On crop lands where farmers use fertilizers to increase crop yields, excess application may lead to higher levels of soil nutrients (Corradini et al., 2019b; Tiecher et al., 2017). However, does fertilizer overuse concur with other environmental threats such as microplastics accumulation? In other words, does a soil managed by a farmer who only loosely adheres to best management practices have more chances of becoming polluted with microplastics? Our data suggests that this is not the case, but this is only the first time this question has been posed and taken into consideration.

Piehl et al. (2018) observed that microplastic pollution of crop lands is higher due to anthropic pressure, even when no plastic covers or microplastic-containing fertilizers are used. Unfortunately, they did not evaluate the relation between high nutrient availability and microplastic pollution as their study area was limited to half a hectare and one

agricultural management regime. Similarly, the increasing body of literature that reports (micro)plastic pollution in crop lands where farmers do use plastic mulch to improve soil conditions also disregards the possible relation between (over)fertilization as an indicator of anthropic pressure —and loose application of best management practices— and microplastics accumulation (see Qi et al. (2020a) for a comprehensive review on the topic). Most certainly, researchers have disregarded this connection because they have addressed only highly productive crop lands where fertilizer use is —more or less— similar across sites.

In this regard, it is important to note that not all nutrient sources —fertilizers and amendments— transport microplastics to soils. There is no evidence of inorganic fertilizers being a source of microplastics pollution. To date, the literature attributes this role only to sludge, compost, and animal dung (Corradini et al., 2019c; van den Berg et al., 2020; Watteau et al., 2018). Further research is needed to expand or revise this claim, as our data points to crop lands as being the most likely soils to receive microplastics, but did not identified the pollution source.

Almost all studies that qualify microplastics found in soils report polyethylene and polypropylene as the most common parent materials of the recovered microplastics (Qi et al., 2020a). Our study follows this trend, and has added polystyrene and acrylates to the list. Our findings confirm those of Piehl et al. (2018) who qualified 12.5% of the microplastics they observed in their assessment of the German farm as polystyrene. And we are the first to report acrylates in soil samples. This polymer is used to extrude fibers so, as the most common microplastic shape we observed in our study was fibers, this relationship could be a possible explanation for why acrylates predominate in our results. Previous studies reporting microplastic fibers in soil samples have not indicated the fibers' polymer type (Corradini et al., 2019c; van den Berg et al., 2020; Zubris and Richards, 2005). This is probably because placing a fiber of less than 1 mm in the ATR unit of an FTIR is an analytical challenge. Researchers studying microplastic pollution of aquatic ecosystems solved this problem by using FTIR microscopes —as we did—, although the detection of fibers along poses challenges (Primpke et al., 2019b), and was a limitation that affected our observations as well.

Although scientific reports on the fate and occurrence of microplastics in terrestrial environments increase every year (compare what was discussed by Bläsing and Amelung (2018) with the topics proposed by Qi et al. (2020a)), the methods researchers use to address the problem remain inadequate (Wang et al., 2020a). The methods we used in this study were no exception. The extraction of microplastics described in section 5.2.4.1 uses a small volume of soil, which may lead to false negatives as explained when comparing our results with those reported by Piehl et al. (2018). We accentuated this problem when we scanned the samples in the FTIR microscope, since the mass of soil from which we extracted

microplastics was even smaller than what we used for the visual sorting. We originally attempted to use the FTIR microscope not only as a tool to qualify the microplastics, but also to quantify them. However, the reproducibility of the method was low as the extraction produced several false negatives. Moreover, fibers challenge the software we used to recognize microplastics, as the kernel convolution algorithm tends to remove fibers because they are only a couple of pixels wide.

Both our results and our limitations emphasize the need for more research on this topic. In our view, the top priority should be to find a reliable method to quantify and qualify microplastics in soils. Although the use of FTIR microscopes is promising and scientists working on microplastics pollution of aquatic environments have contributed largely to its development (Meyns et al., 2019; Mintenig et al., 2017; Primpke et al., 2019b; Primpke et al., 2017; Primpke et al., 2018), a method to concentrate the samples is needed for soils. Little has been done on this facet of the problem. The work of Felsing et al. (2018) with electrostatic separators was a promising breakthrough, but unfortunately discontinued. Along the same lines, finding a valid covariable that correlates with microplastics concentration in soils would alleviate the problem.

The above points aside, this is the first study to characterize the magnitude of microplastic pollution at a regional, multi-land use scale. Our findings challenge some current thinking, and we suspect that our findings may not be unique. Therefore, additional and complimentary studies are needed to increase understanding of the problem's magnitude and environmental consequences.

5.5 Summary and Conclusion

In recent years, scientists have shown increasing interest in studying microplastic pollution of terrestrial ecosystems. However, studies characterizing the problem across multiple land use contexts are missing, and the true scale of the problem has yet to be assessed. We offer a first step in bridging this knowledge gap.

Our results contradict the common belief that microplastics are ubiquitous in the environment, and stresses the role of agricultural activities in the problem. We observed that microplastic pollution occurs more often in managed lands and that microplastics are less likely to reach natural, unmanaged soils, if they reach them at all. Our results indicate that crop lands are the most likely soils to receive microplastics, but interestingly did not provide evidence to identify the pollution source. Additionally, we did not find evidence that connects microplastic pollution with other indicators of anthropic pressure. These findings, in addition to contradicting some common beliefs, highlight current

methodological limitations that are an obstacle to quantifying and identifying microplastics in soils. Finding solutions to these limitations is a research challenge that needs to be addressed. Finally, as this is the first study to report microplastics occurrence on a broad geographical scale, we urge the need for more studies that will contribute data about microplastics in soils under different contexts. Better understanding and eventual management of the microplastics pollution problem will only be possible through increased, real world data.

Chapter 6. Synthesis

6.1 General conclusions

This PhD thesis contributes to the growing body of evidence that identifies and clarifies the sources and dynamics of microplastics in terrestrial ecosystems. It examines the occurrence of microplastics under different land management systems and reveals major pollution sources. It proposes new methods to improve the extraction of microplastics from bulk soil samples and their subsequent identification and quantification and uses the new methods to carry out two environmental assessments.

The outputs of this research advance our understanding of soil pollution and inform scientists about new methods that can be used to identify and quantify microplastics in soils. On the one hand, the thesis offers a proof of concept of a novel method that uses a comparatively cheap and fast instrument to quantify plastic polymers in pollution hotspots (>1% w/w). On the other hand, the inquiries posed by the use of FTIR microscopy triggered the implementation and development of new software to optimize current analytical procedures. Both outputs constitute a general contribution to environmental science that researchers can deploy anywhere to study the accumulation of microplastics in soils. The case studies reported within the thesis emphasized the role of agriculture as the primary cause of microplastic pollution. Moreover, the results of this thesis provide evidence of accumulation patterns of microplastics in soils as a consequence of sludge application, the major source of microplastic pollution in Chile's croplands. Although both study cases examined a local and very particular environment, the insights gained through these studies reflect common environmental scenarios that can also be seen elsewhere. Therefore, the evidence this thesis provides might help to alleviate global plastic pollution.

The following list itemizes the main findings of this PhD thesis:

- Visible to near infrared (vis-NIR) spectroradiometers recording reflectance between 350 and 2500nm identify (classify) and quantify LDPE, PET, and PVC microplastics in soils samples without the need of pre-processing steps. The instrument used, FieldSpec® 3 Analytical Spectral Devices, measures the quantity of plastic particles with an accuracy of 10 g kg^{-1} and a detection limit of ≈ 15 g kg^{-1}. The method outperforms in speed other approaches and bypasses extraction steps by directly quantifying the amount of microplastics present in soil samples. Reported results constitute only a proof of concept since a general implementation requires extensive sets of training data that researchers must generate (adapt or build) for their specific experimental settings. To date, the method can be applied to pollution hotspots.
- The spectral angle mapper algorithm outperforms in accuracy current library search algorithms used to identify microplastics in images from FTIR microscopes.

Parallelization and the cluster computing speeds up the post-processing stage of FTIR analysis. These observations redound in a software implementation that proposes an automatic approach to analyse FTIR images. The new software offers researchers a trustworthy (and transparent) tool to quantify and identify different plastic polymers within environmental samples. Moreover, future users can expand the software to serve other FTIR applications.

- Sludge disposal in agricultural fields leads to microplastic pollution of soils. Accumulation of microplastics in soils increases where wastewater companies dispose of sludge more than once in the same field. In the study area, where soil might receive 40 tons of sludge per hectare every year, sludge constitutes the primary pollution source of microplastics for soils (~$1.36e^9$ microplastic particles per hectare per year).
- Microplastic pollution occurs more often in managed lands (croplands) than in pastures or natural areas. Microplastics are less likely to reach natural, unmanaged soils (natural grasslands), if they reach them at all. Intensive agricultural activities play a key role in driving microplastics into terrestrial ecosystems, even when they do not undergo sludge applications. Other human activities or landscape interventions, such as mining operations, and distance to cities and roads do not correlate with microplastic hotspots in the studied area.

6.2 General discussion

Figure 6.1 summarizes the overall findings of this PhD thesis. The discussion that follows synthetizes the scientific breakthroughs presented in Chapters 2 to 5.

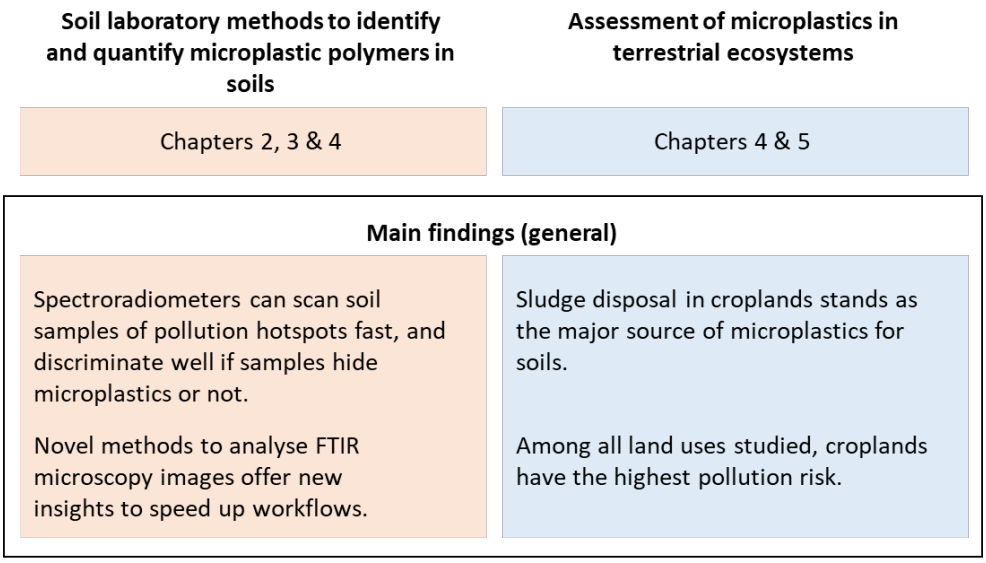

Figure 6.1 *Synopsis of the overall findings of this PhD thesis on microplastic detection and occurrence in soils: detection methods (chapters 2,3 and 4, in orange), and environmental assessments (chapters 4 and 5, in blue).*

6.2.1 New techniques to measure microplastics in soil samples

The first two chapters of this thesis focused on the development and optimization of methods to quantify and classify microplastics in soil samples. Chapter 2 explains that techniques based on vis-NIR spectroscopy could help to screen samples to find microplastic hotspots. The Chapter suggests that the method can be used on-site during field assessments. This constitutes a scientific breakthrough as it allows researchers to directly quantify microplastics in soil samples of pollution hotspots and it stands as a proof of concept for the development of similar techniques. However, the method offers an unrealistic analytical threshold for typical environmental conditions. As a consequence, the method is not ready to be used yet and scientists will still be required to use detailed spectroscopy methods that also suffer from shortcomings (section 1.5). To alleviate this problem, Chapter 3 delves into FTIR microscopy (Agilent Cary 620 FTIR spectrometer), optimizing state-of-the-art methodologies to post-process instrumental readings. The Chapter summarizes a software implementation that reduces the computational resources needed to classify FTIR images. Both Chapters (2 & 3) have reached an audience prior to the publication of this thesis. While Chapter 2 appeared in a scientific journal almost 2 years ago, the Comprehensive R Archive Network (CRAN) released the software described in Chapter 3 at the beginning of 2020.

The early publication of Chapter 2 and 3 play a role in the story line on Section 1.5 about the development of new methods to test soils for microplastics. Science of the Total Environment, a scientific journal, published Chapter 2 as an original research article two and a half years before this thesis was finished. The scientific publisher put the article online at nearly the same time that another study, with similar findings was published. This other study proposed the use of spectroradiometers to measure microplastics in soil samples for the first time (Paul et al., 2019). Differentiating itself from its peer, the article in Chapter 2 offered an alternative perspective. Paul et al. (2019) proposed the novel use of spectroradiometers only to forecast whether or not a soil sample had plastic polymers in it. Instead, the work in Chapter 2 suggested the use of spectroradiometers to quantify microplastics in soils samples. Over the past two years, both studies have triggered new research questions and applications that are founded on the use of spectroradiometers to study the problem of microplastics pollution.

To discuss the scientific contributions of Chapter 2, I must first give a brief summary of recent scientific developments on the subject of the use of spectroradiometers to carry out fast microplastic analysis. In the first breakthrough, Paul et al. (2019) proposed to record the reflections detected in samples with spectroradiometers and to classify the recorded reflection either by principal component analysis or support vector machine regression as plastic-positive or plastic-negative. They observed that the second algorithm, support

vector machine regression, performed better when classifying the spectra. However, both algorithms worked only when soil samples contained between 0.5 to 1% of microplastics by weight. Two facts are troubling about the results of Paul et al. (2019). First, comparable studies carried out at that time deemed these concentrations of microplastics to be unrealistic (Fuller and Gautam, 2016). Second, the accuracy of the proposed dichotomic classification decreased with aged plastics. In other words, the method operated well only for soils highly polluted by young, shiny plastics.

Chapter 2 pushed the boundaries posed by the first study in two ways. First, it tested an alternative algorithm that quantified the polymers in the soil samples. It succeed without lowering the detection limit. In other words, it optimized the analytical procedure by incrementing the information the analysis outputs. Second, Chapter 2 tested the method performance with soils polluted by a mix of different plastic polymers, revealing the potential advantages of the method for challenging environmental situations. Anyhow, the detection limit was still exceeding expected environmental concentrations. The success of using an alternative algorithm pushed scientists to think: Would yet another algorithm optimize the method further?

Renowned scientists evaluated whether or not a supervised machine-learning algorithm could outperform the results of the spectroradiometer's first uses, including the one reported in Chapter 2 (Ng et al., 2020a). They tested a convolutional neural network to classify soil samples polluted with microplastics in the range of 0 to 5% by weight as plastic-positive or plastic-negative . Unfortunately, the algorithm only classified the samples sufficiently for a 2% (w/w) detection limit. The approach failed miserably at discriminating whether a sample held none, low, medium, or high quantities of microplastics, yielding a success rate of 50% in the first case. In other words, the approach could determine whether a sample hid microplastics or not just as well as the flip of a coin. The results enthroned the approach of Paul et al. (2019) and promoted the idea that lowering detection limits below 0.5% (w/w) would require additional steps to concentrate the plastics in a sample. The article I published flirts with this idea. Section 2.4.3 shows how, with the assistance of "appropriate concentration" steps, the detection limit for spectroradiometers could drop as low as 0.7mg of MPs kg^{-1} of soil. A threshold more than 7,000 times lower than what Paul et al. (2019) achieved for bulk readings (5000 mg of MPs kg^{-1} of soil). However, the challenge of discovering how to achieve the "appropriate concentration" of plastics in a sample before analysis alludes us still today.

Two reasons might explain why scientists are reluctant to push forward with analytical methods based on vis-NIR spectroradiometers after accepting that these methods require additional extraction steps. First, the initial use of spectroradiometers to solve analytical problems attracted scientists because it avoided all pre-processing steps that other

methods mandate. Since the need for extra concentration steps stripped away this advantage, scientists quickly lost interest in the technique. Second, once analysts needed to extract microplastics from samples for the method to work, other more ambitious analytical techniques began to compete with spectroradiometers for this task. These other approaches, such as FTIR microscopy, will certainly fit the job better because: a) researchers already use them to study the problem of microplastic pollution, and b) the approaches provide additional information, such as the area of a particle. In other words, why should scientists bother to (develop and) implement a new technique that offers no additional advantages? In addition, an intrinsic characteristic of all methods that use spectroradiometers serves as the third reason that prevents scientists from pushing forward with these methods: All summarized approaches rely on extensive training datasets.

The need for large datasets to train models that predict microplastic concentrations hinders the adoption of methods based on vis-NIR spectroradiometers. The need to record the spectra of representative sample sets for each study location stalls trustworthy and simple analytical workflows. Being aware of this limitation, Qiu et al. (2020b) tested a transfer method to reuse datasets from one location to another. Building on the success of Paul et al. (2019)'s support vector machine regression algorithm, Qiu et al. (2020a) trained a model with samples from soil X to predict microplastics in samples of soil Y. Although they observed an 18% drop in classification accuracy for soil Y with respect to soil X, the accuracy rate rose to almost 80% for a 0.15% detection limit. Qiu et al. (2020a)'s results changed the game as they a) lowered the limit of detection by a factor of 3, and b) suppressed the demand for an infinite number of samples to train the models. Unfortunately, although improved, the detection limit still offers an unrealistic analytical threshold for environmental studies.

Besides the analytical progress on vis-NIR analytical techniques Chapter 2 triggered, two unintended consequences of its publication highlight its worth as proof of concept. Section 1.1 stresses the link between research on microplastic pollution in aquatic and terrestrial environments and elaborates on the idea of water scientists as paladins on the topic. Up to now, methods developed to study microplastic pollution problems in water and sediments has helped soil scientists in their field. Now, for the first time, a method using the spectroradiometer goes in the opposite direction: from soil to water scientists. Piarulli et al. (2020) scanned water samples for microplastics using a hyperspectral camera. Chapter 2's conclusions anticipated this possibility. Piarulli et al. (2020) observed that using vis-NIR spectroscopy offers a rapid method to evaluate water samples without preparation steps. The method fit well with Piarulli et al. (2020)'s purpose, since water samples after filtration do not exhibit the plethora of particles that hide microplastics in bulk soil samples. The hyperspectral camera detected particles as small as 80µm. This knowledge transfer among

disciplines exalts the relevance of interdisciplinary cooperation in environmental sciences and stresses the importance of collaboration to establish the so-called global plastic cycle (Rochman and Hoellein, 2020).

The interpretation of Chapter 2's insights as proof of concept in the development of other, partially related, analytical techniques stands as Chapter 2's second unintended consequence. Following the line of reasoning that justifies Chapter 2, a team of scientists attempted to analyse unprepared sediment samples with FTIR equipment (Hahn et al., 2019). These researchers justified the study by arguing that FTIRs should yield better results than spectroradiometers since the former has better spectra resolution and no overlapping bands. Despite the argued advantages, the authors observed that the method only suited samples with high concentrations of microplastics (>1%). It can predict whether a sample is hiding microplastics or not pretty well, however. One argument the authors wrote to justify their study shines in their introduction. They glimpsed one of the major problems currently facing the study of microplastics in the environment, especially in soils: powerful spectroscopic techniques slow data collection as they increase the time needed for analysis. Chapter 3 took this problem as its motivation, speeding up the analytical time of FTIR microscopy.

To date, FTIR microscopy dominates as *the* spectroscopic analytical technique scientists rely on when they assess field samples (Table 1.2). However, the post-processing of the raw data that FTIR analysis yields hinders the accuracy of FTIR methods (Möller et al., 2020). At this point, scientists have overcome a large number of problems that hold back FTIR spectroscopy from becoming the standard technique (Primpke et al., 2019a; Primpke et al., 2017; Primpke et al., 2018). However, before the release of the R package 'uFTIR' described in Chapter 3, all interventions and advances required several hours of computation. The Comprehensive R Archive Network distributed the first version of the 'uFTIR' software, completely documented in Chapter 3, in March 2020. The software accumulated nearly 1800 downloads as of June 2020. The interest this number reflects stresses the need for better post-processing techniques. The high download number reflects scientists' voracious exploration of every possibility to optimize current post-processing techniques. They test new algorithms to identify polymers (Wander et al., 2020), or directly interact with hardware to speed up the process (Renner et al., 2020). In this regard, the greatest novelty of 'uFTIR' is the implementation of a well-known algorithm in soil science by viewing images from FTIR microscopes as equals to satellite or geo-data images. The idea comes from the studies of Harris (2006), which date from the late 2000s. This cross-over between earth science disciplines water and soil, reflected in 'uFTIR' success, should stand as an example of the relevance of multidisciplinary collaboration in the study of microplastics in the environment.

All in all, the first two Chapters of this thesis reflect part of the transdisciplinary quest that science traverses to find an appropriate method to quantify and classify microplastics in soil samples. Most certainly, the answer to the question of whether or not scientists can develop a perfect method for the analysis of microplastics in complex matrices will not satisfy them. Tandem procedures adapted to specific research goals stand for now as the most plausible alternative (Möller et al., 2020). However, although this solution might work for research, how would it serve monitoring purposes? Even more than research, monitoring needs standardized techniques in order to assess temporal variation properly. Although governments have not yet implemented efforts to monitor microplastics in soils, all the evidence scientists are accumulating will soon push lawmakers to begin the first initiatives. In other words, monitoring efforts should not wait for a perfect method. Section 6.3 offers a brief discussion of this problem.

6.2.2 From theory to practice: the challenges of measuring microplastics in field studies

In addition to the two methods Chapters 2 and 3 offer, this thesis presents a validation of a visual sorting method for soil samples from semi-arid environments. Here, the readers should keep in mind that this thesis presents its chapters in a thematic rather than chronological order to facilitate smooth reading for those who will look for information on a particular subject. However, the chronological sequence, the order in which the chapters were published as research articles that is, makes sense of the methods Chapters 4 and 5 (the environmental assessments) use to test their respective hypothesis. In other words, a cover-to-cover reader might ask: why didn't the author not use the FTIR microscopy method of Chapter 3 in the environmental assessment of Chapter 4? The simple answer: because the research in Chapter 4 took place before Chapter 3. The time gap between these chapters forced the adaption of Chapter 4's method . Therefore, Chapter 4 offers a third method to quantify, without polymer classification, microplastics in soils samples from semi-arid environments.

This thesis presents two studies on microplastic occurrence in soils (Chapter 4 and 5) which provide examples of the difficulties and challenges scientists face when assessing microplastics in the environment. The study laid out in Chapter 4 shares a common denominator with the study in Chapter 2. Both studies were published two years before this thesis was completed. Although Chapter 4 was as the first study that purposely quantified microplastics in the topsoil of fields with sludge applications, it suffers several methodological limitations (see section 4.4.2). The project proposal for the PhD which culminated with this thesis intended for the method proposed in Chapter 2 to quantify microplastics in the topsoil samples in Chapter 4. However, the method did not ultimately

meet the expectations for the detection limit, which rose above realistic environmental concentrations for agricultural soils. Since the method works well only for pollution hotspots, I implemented an alternative method based on what was available to test Chapter 4's hypothesis. The alternative method was visual sorting.

Chapter 4 exemplifies how a simple method to measure microplastics, visual sorting after extracting microplastics by flotation, can be adapted for a particular study condition to serve a specific research purpose. As reported, the data offered a rather pessimistic scenario, since visual sorting provoked false positives (Horton et al., 2017) and, in our case, neglected particles smaller than 2000μm^2 (Chapter 4). Nevertheless, the method was sufficient to answer the study's hypothesis, providing data to support a claim that was up until that moment only theoretical (Nizzetto et al., 2016b). In its internal structure however, Chapter 4 struggles with the method validation. Although visual sorting often helps scientists to identify microplastics and classify them by their morphological characteristics, scientists lack a common framework to carry even the simplest analytical approach (Section 1.5). In this regard and regarding its methodology, the moral Chapter 4 leaves us with supports the idea that the analyst's toolbox should hold a battery of tests to study microplastics in soils (Möller et al., 2020).

Chapter 5 covers the ambitious environmental assessment of microplastics on a regional scale. The methods of Chapter 5 mimic the fragmented and inconsequent links between Chapter 2 and 4. Chapter 5 should have taken advantage of the new algorithm proposed in Chapter 3 to work with FTIR data. However, Chapter 5's methods compiled a hybrid approach that included visual sorting. As indicated in Section 5.4, a stand-alone FTIR analysis would have implied an overall detection limit far above the expected concentrations. The FTIR analysis reported in Chapter 5 required small sample volumes, which increased the method's detection limit. The inclusion of visual sorting ameliorated this problem. Nonetheless, the FTIR method limitation resulted in a lower number of FTIR positive samples, even for samples pre-evaluated for microplastic pollution. Regarding the FTIR analysis, the methodological limitations correlated with the procedure used to extract microplastics from the samples rather than with the instrumental measuring itself (as in the case of Chapter 4). How do other researchers extract microplastics and avoid this problem? Once again, there is no standardization. This time, however, Chapter 5 struggled with extraction as it attempted for the first time an extract-and-measure approach without transferring or selecting suspicious particles from a subsample batch. The motivation of this novel idea is grounded in a FTIR limitation. During FTIR analysis, researchers pick up particles from a subsample of a batch of 'suspicious plastic particles' to inspect them in a FTIR instrument. This procedure generates two problems: a) the subsample is biased to larger particles since researchers need tweezers to hold the suspicious particles, and b) the subsample is transferred from the filter that collected it to the tray of an FTIR machine with

dubious consequences. Chapter 5 is purposeful. It departs from mere repetition and attempts to evaluate all particles despite their size in a new approach. In its attempt, Chapter 5 exposes the trade-off of doing so: the small volume of the sample the analyst takes for the analysis raises the detection limit generating false negatives. In this regard, Chapter 5 reminds us of the need to develop appropriate concentration steps for more direct analytical protocols. A reminder that echoes Chapter 2's moral.

Another remainder this thesis shapes is the lack of open, and reproducible research methods. In this regard, the lack of public spectral libraries to use as references stands as the most prominent example. All spectroscopic methods need reference libraries to match what the instrument reads with a known spectrum. However, researchers have to date only one open library freely available (Primpke et al., 2018). This is inconvenient as, for example, bioplastics —among other polymers— are underrepresented (Fojt et al., 2020). This analytical shortcoming should be addressed by future research efforts.

In Summary, the experience of measuring microplastics in soil samples after four years of experimenting with possible analytical pipelines indicates that a unique method seems inappropriate to fit all study questions and hypotheses. The optimal path seems to compile different standardized methods to provide researchers enough flexibility to study challenging problems. This approach will allow for the appropriate comparison of different research efforts. This reflection relates to the moral of Chapters 2 and 3, which I expressed at the end of Section 6.2.1.

6.2.3 New evidence provided to close the global plastic cycle

This thesis offers new evidence on microplastic occurrence in the topsoil of a semiarid region in an upper-middle income country. It reports that sludge disposal in agricultural lands is the predominate source of microplastics (Chapter 4). This thesis also points out that among all land uses, croplands are at the greatest risk from microplastic pollution even when farmers do not fertilize with sludge nor use plastic mulches (Chapter 5). The insights of this thesis support the idea that plastics are ubiquitous in ecosystems, supporting the global plastic cycle theory. As a result, the contributions that this thesis makes fit perfectly into this time and place. Consequently, in this section, I discuss these contributions within their temporal context.

Recently, scientists realized that the problem of plastic pollution transcends the limits of a single discipline or the boundaries of a particular ecosystem (Rochman and Hoellein, 2020). While the idea of the global cycle of plastics grows along with the evidence that supports it, relevant information gaps remain unapproached (Rillig, 2020). Information gaps grounded in methodological limitations (Section 6.2.1 and 6.2.2), and in the novel relevance of the

problem. Although concerns about plastics in the environment began in the early 1970s (Carpenter and Smith Jr, 1972), their propagation to disciplines other than oceanography did not start until a decade ago (Rillig, 2012). Researchers had studied only tangentially ideas such as the aerosolization of microplastics as an ocean to land transportation process (Zhou et al., 2018), or the ingestion and excretion of microplastics by humans (Schwabl et al., 2019). Scientific journals accumulate new evidence every year and publish new breakthroughs, such as the uptake of microplastics by plants (Li et al., 2020b). However, the ubiquity of microplastics enlarges the problem and offers, as time goes by, a plethora of new dark corners that scream for enlightenment.

The original publication of the study Chapter 4 transcribes sheds light on an unknown problem of its time: Does sludge disposal in farmlands provide microplastics to soils? (see section 1.4) Chapter 4's evidence expanded the technical discussion about sludge dispositions on three fronts: 1) waste management, 2) nutrient recovery, and 3) environmental pollution. Scientists questioned sludge disposal in soils even before learning that sludge carries microplastics to soils (Liu et al., 2011). The new evidence about unintended transport of microplastics to soils by sludge disposal only added a new argument to a long list of motives some scientists use to discourage sludge application in croplands (Mohajerani and Karabatak, 2020). Chapter 4's conclusions contributed to defeating a common argument that supporters of sludge applications made. Even now, supporters of sludge disposal in croplands say that the benefits of nutrient recovery outclass the environmental drawbacks whenever application rates are moderate (Seleiman et al., 2020). They claim that evidence suggests plant uptake of organic contaminants and heavy metals does not cause a significant hazard to plants and rarely surpasses environmental thresholds. They argue that other potentially hazardous compounds decompose or volatilize at fast rates which decreases potential leaching. However, since plastics, even bioplastics, do not degrade in soils except under very special circumstances (Fojt et al., 2020; Zhang et al., 2020d), Chapter 4's conclusions present a breaking point for that line of reasoning. Since its publication, other studies have supported Chapter 4's conclusions: microplastics accumulate as a result of long-term sludge applications (see Table 1.2) and only one application is needed to pollute soils to measurable levels (Cattle et al., 2020; Crossman et al., 2020; Liu et al., 2018; van den Berg et al., 2020; Zhang and Liu, 2018; Zhang et al., 2020b). This knowledge challenges what promotors of nutrient recovery have argued about inorganic contaminants in sludge. Since microplastics concentrations increase over time, to use soils as microplastic sinks could generate a time bomb for diffuse pollution of microplastics to waterbodies and provoke disruptions in soil biota. The discussion could extend to compost application as well, since incipient evidence indicates compost might source microplastics to soils (Cattle et al., 2020). The new standoff between nutrient recovery and soil pollution by sludge disposal or compost applications might cause policymakers to change sides. However, the ban on sludge disposal in farmlands would

imply economic consequences. In other words, what would be the consequence for circular economy and energy consumption if sludge applications to soils were banned? How would the potential demonization of sludge disposal in farmlands affect waste management in low and lower-middle income countries where other technologies to deal with sludge might be cost prohibitive? (landfills disposition, or ignition). Certainly, as the picture of microplastic pollution becomes more clear, political compromises rather than technical reasons will answer these questions.

Thus, Chapter 4's conclusions contribute to the big picture of plastic pollution that the scientific community has sketched. Estimations on the quantity of microplastics that reached soils through sludge disposal came out right after the first concerns about the occurrence of microplastics in soils surfaced. Estimating an average per-capita loading between 0.2 and 8 mg of microplastics per hectare each year, researchers suggested that sludge disposition might source between 63,000 to 430,000 and 44,000 to 300,000 tons of microplastics annually to soils in Europe and North America, respectively (Nizzetto et al., 2016b). Within the global dynamics of (micro)plastic pollution, these numbers cause alarm for environmental scientists as the theoretical retention rate for microplastics in soils ranges between 16 to 38% (Nizzetto et al., 2016a). Thus, a great proportion of the microplastics that ends up in soils undergoes offsite translocation. The fact creates an efficiency paradox. When water scientists revealed the occurrence of microplastics in water, they realized that wastewater ranks first among the possible entry ways of microplastics to superficial waterbodies (Talvitie et al., 2015). They highlighted the relevance of having efficient wastewater treatment plants to counteract the problem. In due time, engineers increased the efficiency of wastewater treatment plants, which now generate low-microplastic effluents (Sol et al., 2020). As a consequence, microplastics now remain trapped in the sludge (Frehland et al., 2020). However, wastewater treatment plants dispose of sludge on agricultural fields where only a portion of the trapped microplastics remain on-site (Chapter 4, Crossman et al. (2020) and Wang et al. (2020c)). What do all these interconnections mean to the overall efficiency? Given the intricate links that make up the global plastic cycle, what could constitute a good mitigation measure?

Along with sludge disposal, scientists should bridge other knowledge gaps in our understanding of microplastic pollution prior to proposing new mitigation measures. Researchers recognise sludge disposal as one of the major entry ways of microplastics to soils (Sol et al., 2020). However, they lack information about the concentrations of these pollutants in different land uses and management scenarios that do not necessarily undergo sludge applications, such as natural grasslands, pastures, and croplands without sludge applications (Piehl et al., 2018). The scientific quest laid out in Chapter 5 makes an attempt to try and begin to address this problem. The lack of data on realistic concentrations of microplastics in the environment constitutes one of the biggest problems to date. It hinders

research and regulations (see section 6.3.2). Without knowing the size of the problem, policymakers would always face difficulties in allocating resources and mitigating the problem. Section 1.3 discusses what science knows so far about the sources of microplastics and their interconnections. The current conceptual framework does not question the whereabouts of microplastics in soils (Kumar et al., 2020). It considers soils only as a partially stationary sink for microplastics, while it ties the problem mostly to agricultural soils. Recently, a new paradigm began to take over. Both the first and the second most prominent journals on multidisciplinary sciences, Nature and Science, have published opinionated articles that advocate for a new problem conceptualization. The new concept needs to address the plastic problem as an environmental cycle of anthropic origin (Rillig, 2020; Rochman and Hoellein, 2020). The motivation of Chapter 5 supports this new idea since it questions soil microplastic pollution from a comprehensive perspective. In other words, Chapter 5's conclusions support the new conceptual framework by wondering about microplastic pollution in all soils, despite the land use.

Chapter 5 supports the idea that soil pollution is a ubiquitous problem in terrestrial environments. Researchers have emphasized this several times already (Scheurer and Bigalke, 2018; Thompson et al., 2004), although hard evidence of their claims has yet to be collected. Now, this idea redounds in a new conceptualization for the problem of plastic pollution, presenting it as a global cycle that originates with us, humans (Rillig, 2020; Rochman and Hoellein, 2020). An idea tailored by its time and place, as its correlates well with the broader paradigm of the Anthropocene: the idea that now strives to dominate our understanding of environmental problems (see Zalasiewicz et al. (2016) for a deep and illustrative discussion about plastics, soils, and the Anthropocene). To put it differently, the evidence in Chapter 5 constitutes another brick in the wall that will cement our understanding of human interventions as one predominant force that modifies earth landscapes and ecosystems.

Chapter 5 leaves one big question unanswered: as croplands are not treated with sludge nor used plastic covers, from where does the microplastic particles come from? Unfortunately, the study did not provide a hint about the potential sources. To date, scientists propose compost and plastic coated fertilizers and agrochemicals as potential sources (Qi et al., 2020a). This potential entry ways, together with aeolian transport and deposition, should be studied with care in the future.

In summary, the studies discussed in Chapters 4 and 5 contribute new perspectives to the global plastic cycle, supporting and becoming part of current trends in environmental research. Although the studies constitute scientific breakthroughs, they only concern a minor part of the broader problem of plastic pollution. They report only on microplastics and they involve either one pollution source (Chapter 4) or provide expected

concentrations in the topsoil of a particular world region (Chapter 5). The fact that research on microplastics in terrestrial environments started less than a decade ago, only stresses that a long road extends ahead of us. A road we must traverse to accumulate enough understanding and guide our actions.

6.3 Implications and recommendations for policymakers and land managers

Warning about microplastics in terrestrial ecosystems (Rillig, 2012), scientists triggered the study of the transport processes of microplastics across different matrices and their interactions (Nizzetto et al., 2016a). By the time they started, water scientists had done a tenacious job informing the public and politicians about the threats and problems that microplastics pose to aquatic environments (Henderson and Green, 2020). On the one hand, citizens were participating in activities to contribute solutions, such as citizen science initiatives to size up the problem (Gaibor et al., 2020; Lots et al., 2017). On the other hand, policymakers were promoting the first bans of potential sources (Herberz et al., 2020). However, today's expansion of the pollution problem from aquatic to terrestrial ecosystems complicates the development of regulatory measures aimed at mitigation.

Although the cycle of microplastics through the environment baffles scientists with its unknowns, the public perceives a rather simplistic scenario. Citizens and policymakers connect plastic pollution with disposable plastics the most (Henderson and Green, 2020). Regarding microplastics, citizens recognize microbeads from personal care products as a main pollution source (Anderson et al., 2016). These suppositions have led to the banning of single-use plastics and microbeads. However, these measures will not suffice. First, banning single-use plastics is not good for the environment (Herberz et al., 2020). Life cycle assessments show that while GHGs emission due to the production cycles of plastics would be somewhat reduced (~5%), other toxic substances would increase as a result of the production processes of substitute products. Second, evidence suggests that microbeads from cosmetics and detergents are not to blame for the largest amount of microplastics emitted to seas (Duis and Coors, 2016). Instead, evidence suggests that plastic microfibres of diverse compositions and sources (Table 1.1 and 1.2) rank first in frequency among microplastics in the environment (air (Dris et al., 2016), soil (Wang et al., 2020a), sea (Reineccius et al., 2020), freshwater (Valine et al., 2020), fauna (Carlin et al., 2020), and overall (Xu et al., 2020)). Although not unique, the largest source of plastic microfibers in the environment is wastewater treatment and sludge disposal (see section 1.3, and Chapter 4). Mitigation measures should primarily aim to strike at these large pollution sources. This does not mean that mitigation should concentrate only on reducing the dispersion of microfibers in the environment and neglect the other shapes and sources of microplastics .

However, the dispersion of microplastics through sludge disposal cannot continue to be ignored by regulations (see Nizzetto et al. (2016b) discussion about the matter). The lack of awareness and the lack of progress policymakers can make to prevent microplastics' dispersion through sludge disposal can be partially justified. This type of environmental degradation offers a complex problem: policymakers cannot ban plastic microfibres as easily as they ban single-use plastics or any other easily identifiable pollution source such as agricultural plastic mulch.

The problem plastic microfibres pose is multifaceted. There is no direct (eco-friendly) alternative to the use of plastic fibres in the textile industry. Plastics provide cheap materials allowing a huge segment of the population access to (reasonably) affordable clothes. Moreover, offering natural fibres as a possible substitute to plastic throws other problems into the mix: water scarcity, soil loss, and ecosystem deterioration (La Rosa and Grammatikos, 2019). Since plastic fibres cannot be banned for now, they will continue to end up in the environment. Detached microfibres will keep entering waterbodies wherever human settlements do not connect to wastewater treatment plants or the fibres will be caught up in the sewage sludge of the wastewater treatment plants and keep polluting the environment. The reader might glimpse the true dimension of the problem if she/he: (1) acknowledges that no country connects all its population to wastewater treatment plants (OECD, 2019), and; (2) recognizes the size of the problem that sludge disposal poses: current estimations predict that more than 50% of the sludge generated in Europe and North America ends up on agricultural lands (Hurley and Nizzetto, 2018). Even if waste managers decided to incinerate all the sludge, land managers would have to provide alternatives to support organic matter accumulation (see 4 per 1000 initiative). Thus, how could governments prevent soil pollution by sludge disposal while at the same time replacing sludge as carbon source?

When applied to soils, sludge partially contributes to soil's organic matter. So in effect, sludge application is really more of a way to avoid incineration or landfill disposal than a way to raise soil organic matter. However, sludge application does matter for nutrient recovery. For example, direct application of sewage sludge amendments in order to supply soils with phosphorus demands less energy than the application of mineral fertilizers or phosphate fertilizers derived from sewage sludge (Linderholm et al., 2012). The trade-off that nutrient recovery poses asks whether sewage sludge applications as fertilizer sources would be possible without polluting the ocean while doing so. In other words, is there any sludge application threshold for soils that allows nutrient recovery at low energy costs without the off-site transport of microplastics? Would that threshold guarantee near-zero in-site detrimental effects?

A compromise between environmental health and waste management will most certainly require a definition of maximum tolerable concentrations of microplastics in the environment. To define these thresholds, policymakers should consider the diverse substances that compose microplastics (Table 1.1) and understand that the word 'microplastics' refers to a plethora of possible pollutants. Should all thresholds be the same for all possible polymers? This question highlights the need to extend our current knowledge. Scientists have collected evidence of microplastics' hyperaccumulation of other pollutants only for some polymers (polyethylene and polypropylene, see section 1.4) (Ramos et al., 2015; Yan et al., 2020; Yang et al., 2019a; Zhou et al., 2019). Ecotoxicological studies have evaluated mostly polystyrene and light density polyethylene (Cao et al., 2017; Jiang et al., 2020). Studies that address the transport of microplastic particles through the soil profile have focused only on laboratory conditions, including only a limited selection of polymers (polyethylene and polyester) and shapes (microbeads, fibres) (Keller et al., 2020; O'Connor et al., 2019; Yu et al., 2019). And what about bioplastics? Scientists have just started to wonder and gather information about their fate and transport in the environment (Fojt et al., 2020; Shruti and Kutralam-Muniasamy, 2019). Along with composition, the proposal of environmental thresholds should also consider the size of microplastics. The proposition of a definition for microplastics made by water scientists might not fit current needs. Water scientists define 'microplastics' as plastics less than 5mm *"to focus the microplastic discussion on possible ecological effects other than physical blockage of gastrointestinal tracts"* (Arthur et al., 2008). Is this definition appropriate for soils? Movement thorough the soil's pore space happens only for plastic particles less than 500µm (Keller et al., 2020). And earthworms guts malfunction or can become blocked when plastic particles as small as 1µm are present, so what happens with the particles are as large as 5mm (Jiang et al., 2020)? Scientists should search for answers to these questions and build up our comprehension of the big picture of microplastics in the environment, which is fundamental to the task of proposing comprehensive mitigation measures.

Mitigation measures should reduce the loads of microplastics to wastewater to prevent the dispersion of these pollutants through sludge disposal. The quantities of microplastics in sludge rise to an unmanageable scale at wastewater treatment plants. Therefore, mitigation measures should focus on whatever it is that leads microplastics to wastewater. For example, researchers might wonder how to optimize the production and use of plastic fabrics to reduce the detachment of microfibres. The quest to find solutions will reunite several disciplines. Product designers and engineers could offer partial solutions by optimizing washing machine filters and processes (Schöpel and Stamminger, 2019). An optimization shyly explored to date (Cesa et al., 2020). However, how many households worldwide own washing machines? With this example, I would like to stress that potential solutions, when faced with reality, will always demand extra measurements and conjoint optimizations coming from different research fields. To reduce the quantity of microplastics

that reach wastewater treatment plants, researchers must look for several 'upstream' pollution sources in order to begin optimization efforts (Cai et al., 2020). With this, material scientists will play an important role for years to come. The improvement of microfibres to increase their shear stress constitute, for example, a technology that might alleviate the problem at its very source (De Falco et al., 2019). However, no matter how good the advances that scientists achieve are in the coming years, the problem will not be solved without the involvement of policymakers.

Recently, governments began to propose the first regulations to reduce microplastic pollution and its potential environmental consequences (as described in Section 1.4). The ubiquity of the problem and the complex interactions between microplastic sources and sinks demand proper monitoring. Only by monitoring the environment will governments be able to properly allocate the resources needed to mitigate the problem. In this regard, authorities should define application thresholds or maximum annual loads for soils and maximum contents of microplastic particles in sludge and compost. Unfortunately, the lack of appropriate and standardized sampling and analytical protocols currently hampers the implementation of both routine monitoring programs, and application thresholds. For example, the European Parliament failed to regulate the emission of microplastics from wastewater treatment plants in the Legislative Resolution of 12 February 2019 (TA/2019/0071). The Resolution captured how the lack of standardized methodologies impeded the proposal of mitigation measurements. One of the amendments reads *"The Commission Joint Research Centre should develop parameters and measurement methods to identify the presence of microplastics and pharmaceutical residues in reclaimed water"*, a goal not yet met. The Parliament has not yet proposed regulations targeting soil pollution. Given the current analytical challenges (Sections 1.5 and 6.2.1), it is reasonable to predict that the problem of truncated monitoring initiatives will impede future attempts of the Parliament to protect terrestrial environments as it currently does for aquatic environments.

In summary, the design of adequate prevention and mitigation strategies to lessen the impacts of microplastic pollution challenges environmental scientists and policymakers. Especially now that the problem has been shown to be a complex environmental cycle of plastics traveling from one ecosystem to the next, causing all kinds of trouble in their wake (Rochman and Hoellein, 2020). Prevention alternatives fade as plastics lay at the very core of our daily lives. Mitigation options shrink as inequality, lack of technology, and investment in research hampers new developments in most countries worldwide.

6.4 Research challenges and future research directions

This PhD work contributes to the improvement and development of methods to quantify and classify microplastics in soil samples and, by deploying these methods, it has contributed to our understanding of the global plastic cycle. While this research adds to the growing corpus of research on plastics in the environment, scientists should explore a number of topics in order to close the gaps that prevent the design of appropriate mitigation strategies, namely:

- To revise and to adjust the formal definition of microplastics. Currently, the upper size threshold for microplastics works only for water scientists and does not reflect the behaviour of microplastics in soils. For example, if soil scientists want to emphasize transport processes, they should lower the upper threshold for microplastics' size (i.e. they should study smaller particles). Any future definitions must adapt to the current framework, moving with the global plastic cycle paradigm.
- To improve analytical methods regarding polymer recognition and quantity estimations. Accurate polymer recognition stands as the *sine qua non* condition to identify pollution sources, the essential step to propose effective mitigation measures. Improved methods should provide quantities by polymer and not mere totals. Moreover, analytical techniques should quantify microplastics in a consistent unit, whether as mass, volume, or count.
- To standardize sampling procedures. As discussed in Chapter 5, different authors have used different sampling strategies. Researchers should revise them to evaluate their advantages and disadvantages for the analysis of microplastics, taking into account different study and monitoring purposes.
- To recognize the magnitude of the plastic pollution problem. New field assessments should provide data to close the knowledge gaps that prevent the recognition of pollution sources and sinks. The size of the problem and its ubiquity should be evaluated to support realistic laboratory experiments. Scientists should combine the theory of the global plastic cycle with data to propose adequate mitigation and remediation measures. In this regard, field assessments should include spatial and temporal dynamics of microplastic concentrations in soils.
- To evaluate the effects of different land management strategies and edaphoclimatic conditions on the fate of microplastics in soils. Evidence suggests that soil structure, irrigation, organic amendments, and land use among other soil and field conditions affect soil microplastic concentrations and off-site transport. Funding agencies should support data collection for these purposes.

- To evaluate the effects that microplastics can cause on soil microorganisms. *In situ* evaluations should study the effects on different soil organisms across different steps in the food chain.
- To understand the fate of microplastics in soils. Scientists should study the fate of microplastics within the soil profile regarding soil pore space occlusion, aggregate stability, water availability, pollutant adsorption, etc. Studies on this topic are yet incipient.
- To identify the contribution of different pollution sources and its transport mechanisms.
- To reveal microplastics offsite transport processes and migration to aquatic environments.

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Summary

Plastics serve as versatile and malleable matrices that manufactures can easily manipulate in an effort to develop new technologies to benefit society. These new technologies allow manufacturers to produce products that customers can use either a single time or for more than 50 years. Due to its great versatility, industries across all sectors use plastic at some point during the production process or include plastic in the final products. This great versatility comes with a high demand and use of plastic. This produces a complex scenario with regards to plastic disposal. According to the best educated guess, nearly 24 million tonnes of plastics that were improperly disposed of ended up in terrestrial ecosystems in 2010 alone. Since then, human demand for plastics and plastic consumption have steadily risen and increased to roughly 4 times what they were in 2010 (they reached 51.2 million tonnes in 2018). The implication reads: pollution and the transfer of plastics to ecosystems have worsened over the last 10 years.

Large plastic chunks threaten the environment mainly through direct ingestion by wildlife. Smaller plastics measuring less than 0.5cm threaten the environment not only by their inherent toxicity but also by transporting other pollutants as they themselves move through the soil to other sites. Scientists named these smaller plastic pieces microplastics. Because microplastics constitute a potential threat to soil biota and water or wind might spread them through the environment by transporting them off-site, researchers have finally started to study the occurrence of microplastics in soils. Although researchers have gathered evidence on the occurrence of microplastics in soils as well as the effects that high concentrations might have on soil biota, a few methodological problems have hampered the proposal of new mitigation measures.

To date, researchers have failed to articulate and standardize a reliable soil test to identify different plastic polymers and quantify particles in soil samples. In general, if we cannot effectively measure something, we cannot study it. For this reason, comprehensive data on the occurrence of microplastics in different soil environments or under different land uses is lacking. The ramifications of this lack of data can be seen in the unrealistic laboratory tests where researchers tested the effects of exaggerated concentrations of microplastics on soil biota. Thus, this thesis aims to contribute to the growing body of evidence that identifies and clarifies the sources and dynamics of microplastics in terrestrial ecosystems. It intends to shed light on the occurrence of microplastics across different land uses and to reveal major pollution sources. To do so, it proposes new approaches to detect and quantify plastic polymers in soil samples and then uses these new approaches in a case study from Central Chile.

In Chapter 2, we evaluated a handheld spectroradiometer working in the near infrared range (350-2500nm) as an instrument to directly measure microplastic concentrations in soil samples. The Chapter reads as a proof of concept. The results suggest that vis-NIR techniques are able to identify and quantify LDPE, PET, and PVC microplastics in soil samples, with a 10 g kg^{-1} accuracy and a detection limit ≈ 15 g kg^{-1}. The method stands out since it allows researchers to process samples fast (2 min), avoids extraction steps, and can directly quantify microplastic quantities. As a proof of concept, the proposed approach has motivated the development of other similar methods intended to measure soil and water samples.

The approach proposed in Chapter 2 worked only for pollution hotspots. We wanted to add a method to the toolbox that scientists could use to detect and classify even small amounts of microplastic particles made of different polymers in soil samples. In the original research plan, this 'detailed' method was to be used in the environmental assessment of Chapter 5. µFTIR analysis would have allowed this. However, the current available software lacks the functionalities needed to process large amounts of data and lacks the ability to take the most out of µFTIR spectroscopy images. Therefore, in Chapter 3, we present a new software released by The Comprehensive R Archive Network that was designed to be used within the R environment and optimizes current procedures by deploying a novel algorithm to process spectroscopy images (https://CRAN.R-project.org/package=uFTIR).

In Chapter 4, we studied the effect of long-term sludge applications on the accumulation of microplastics in soils. To do so, we sampled soils in Chile's central valley. Like many scientists studying microplastics in soils, we suffered methodological limitations when we analysed the samples. At the time we carried out the analysis, the µFTIR spectrometer was not available yet and the approach proposed in Chapter 2 did not detect microplastics in the concentrations we expected to find. Therefore, in Chapter 4, we validated an extraction method that uses flotation to isolate microplastic particles from bulk soil samples and then sorted the plastics by their morphology. The study results indicated that the number of microplastic particles increased over time in the soils that received long-term applications of sludge (from 0 to 3.500 microplastic particles per kilo of soil). The study stresses the relevance of sludge as a driver of soil microplastic pollution. It was the first evidence of the role of sludge disposal as a pathway for microplastics to enter into soils.

In chapter 5, we look at the occurrence of microplastics in soils under different land uses. The motivation behind this chapter is in line with the current idea of a global microplastic cycle. The study aimed to assess the presence of microplastics in the topsoil of land exposed to different land management systems at a regional level in Chile's central valley. To do so, we used the sorting method validated in Chapter 4 and classified microplastics using µFTIR, processing FTIR images with the software described in Chapter 3. Results showed that

croplands and pastures were exposed to microplastic pollution, while this type of pollution seldom occurred in rangelands and natural grasslands (both not managed). The study emphasized the role of agriculture in spreading microplastics through the environment. As the first study that has reported the occurrence of microplastics in soils on a broad geographical scale, it underscores the need for more studies that offer actual monitoring data concerning microplastics in soils.

The combination of chapters in this thesis contributes to the growing body of evidence on microplastics in terrestrial ecosystems. The results rank human activities, such as agriculture and waste management, as the first factor that contributes to the direct pollution of soils. It provides new insights that will help to bridge some of the knowledge gaps related to analytical procedures. This thesis also reflects on methodological limitations, stressing the need of proper soil tests that can help quantify and classify microplastics in soils. Taken together, the chapters of this thesis support the idea that an analyst's toolbox should comprise versatile but standardized soil tests in order to study microplastics in the environment. All in all, this thesis describes three methods that can be used to quantify and qualify microplastics and uses them to report temporal (Chapter 4) and spatial (Chapter 5) variations of microplastics in soils. It is my honest opinion that the data gathered using these methods will support the concept of the global plastic cycle. A concept that will help scientists to communicate their concerns to a broader audience. It will also help policymakers to craft mitigation strategies that buffer the impacts of human activities on the environment.

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Fabio Corradini, Wageningen.

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Fabio Alfonso Corradini Santander was born on August 08, 1987, in Santiago, Chile. He studied agricultural engineering and obtained a master's degree in soil and water management at the University of Chile. Before the PhD he worked in private and public companies classifying soils, and designing and performing experimental trials to study soil fertility and fertilizers. Since 2016, he works at Chile's Agricultural Research Centre, as soil scientist.

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- Environmental research in context (2017)
- Masterclass Git, GitHub and Markdown in a R-Environment (2018)
- Model training for scenario analyses: River export of nutrients from land to sea (2018)
- Research in context activity: 'Initiating and organizing expert stakeholder meeting on Microplastics in soil: the impacts of sludge applications on Chilean central Valley' (2019)

Other PhD and Advanced MSc Courses

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- Workshop the Choice: Taking charge of your performance, Wageningen University (2017) Bayesian statistics, Graduate Schools PE&RC and WIMEK (2017)
- Geostatistics, PE&RC Graduate School (2017)
- Scientific writing, Wageningen Graduate Schools (2019)
- Brain-friendly Working & Writing, Wageningen Graduate Schools (2019)
- World soils and their assessment, ISRIC (2019)
- Geocomputation using free and open source software, Graduate Schools PE&RC and WIMEK (2019)

Other Societal impact Activities

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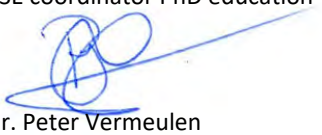
- *Sewage sludge: the main carrier of microplastics to agricultural soils*. 17th International Conference on Chemistry and the Environment, 16 –-20 June 2019, Thessaloniki, Greece

SENSE coordinator PhD education



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