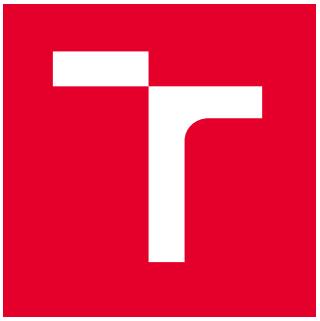

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VYSOKÉ UČENÍ TECHNICKÉ V BRNĚ

FACULTY OF CHEMISTRY

FAKULTA CHEMICKÁ

INSTITUTE OF CHEMISTRY AND TECHNOLOGY OF ENVIRONMENTAL PROTECTION

ÚSTAV CHEMIE A TECHNOLOGIE OCHRANY ŽIVOTNÍHO PROSTŘEDÍ

INFLUENCE OF BIOPLASTIC BIODEGRADATION ON SOIL PROPERTIES

VLIV BIODEGRADACE BIOPLASTŮ NA PŮDNÍ VLASTNOSTI

MASTER'S THESIS

DIPLOMOVÁ PRÁCE

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VEDOUCÍ PRÁCE

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Zadání diplomové práce

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Zadání diplomové práce:

1. Literární rešerše na téma bioplasty, jejich degradace, benefity a rizika spojená s jejich využitím, půda a její ekosystémové funkce.
2. Provedení květináčových experimentů zkoumající vliv bybraného biodegradabilníhi bioplastu na růst rostliny a vlastnosti půdy.
3. Analýza půdy a rostliny.
4. Vyhodnocení a diskuze získaných výsledků.

Termín odevzdání diplomové práce: 8.5.2023:

Diplomová práce se odevzdává v děkanem stanoveném počtu exemplářů na sekretariát ústavu. Toto zadání je součástí diplomové práce.

Bc. Veronika Stanislavová studentka	prof. Ing. Jiří Kučerík, Ph.D. vedoucí práce	prof. Ing. Jiří Kučerík, Ph.D. vedoucí ústavu

V Brně dne 1.2.2023

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děkan

Abstract

Biodegradable plastics are gaining popularity as a desirable material in the market, particularly in the agricultural sector, where they are used as coatings and mulch films due to their favourable properties. This study investigated the impact of poly-3-hydroxybutyrate (P3HB), an intracellular bacterial polymer, on various soil properties, including physical, chemical, and biological aspects, to assess its potential in agricultural use.

Results showed that the P3HB had a negative effect on the soil dry matter content, aboveground biomass, and a slight decrease in soil pH. Moreover, the activity of selected enzymes was significantly enhanced when P3HB applicated solely or even more increased when combined with a specific amendment. The results varied depending on the amendment; the effects were negative, neutral or positive. The thermogravimetry analysis was used to assess the rate of P3HB biodegradation and the influence of P3HB on organic matter and water retention. It was found that the presence of amendment influenced the degradation rate of P3HB and other organic matter in the soil.

Furthermore, the application of P3HB into the soil leads to the inhibition of plant (*Zea mays L.*) growth. The additions of amendments to soil prior to the application of P3HB improved this effect only marginally. It is concluded that biodegradation of P3HB supported the activity of the microbial community, which could eventually negatively influence the availability of essential nutrients in the soil. The thermogravimetric analysis demonstrated that the incorporation of organic amendments promoted the biodegradation of P3HB. Moreover, the results indicate that a combination of P3HB and biochar could improve soil water retention.

Keywords

Biodegradable plastics, biodegradation, P3HB, organic amendments, soil properties, enzymatic assays, soil respiration, microbial activity, thermogravimetry, mass loss.

Abstrakt

Biodegradabilné polyméry sa na trhu stávajú čoraz populárnejšími, a to najmä v poľnohospodárstve, kde sa vďaka svojim priaznivým vlastnostiam používajú ako nátery a mulčovacie fólie. V tejto štúdii bol skúmaný vplyv poly-3-hydroxybutyrátu (P3HB), vnútrobunkového bakteriálneho polyméru, na rôzne vlastnosti pôdy vrátane fyzikálnych, chemických a biologických parametrov s cieľom posúdiť jeho potenciálne využitie v poľnohospodárstve.

Výsledky ukázali, že P3HB mal negatívny vplyv na obsah sušiny v pôde, nadzemnú biomasu a spôsobil mierne zníženie pH pôdy. Okrem toho sa aktivita vybraných enzýmov výrazne zvýšila, keď sa P3HB aplikoval samostatne alebo došlo k ešte väčšiemu nárastu v kombinácií s niektorými pôdnymi doplnkami. Výsledky sa líšili v závislosti od použitého pôdneho doplnku. Účinky boli buď negatívne, neutrálne alebo pozitívne. Na posúdenie miery biodegradácie P3HB, vplyvu P3HB na organickú hmotu a zadržiavanie vody sa použila termogravimetrická analýza. Bolo preukázané, že prítomnosť organických doplnkov ovplyvnila rýchlosť degradácie P3HB a iných organických látok v pôde.

Okrem toho aplikácia P3HB do pôdy viedla k inhibícii rastu rastlín (*Zea mays L.*). Pridanie zmien do pôdy pred aplikáciou P3HB tento účinok zlepšilo len okrajovo. Dospelo sa k záveru, že biodegradácia P3HB podporovala mikrobiálnu aktivitu, čo mohlo v konečnom dôsledku negatívne ovplyvniť dostupnosť esenciálnych živín v pôde. Okrem toho výsledky z termogravimetrickej analýzy naznačujú, že kombinácia P3HB a biouhlia by mohla prispieť k lepšiemu zadržiavania vody v pôde.

Kľúčové slová

Biodegradovateľné plasty, biodegradácia, P3HB, pôdne doplnky, pôdne vlastnosti, enzymatické testy, respirácia pôdy, mikrobiálna aktivita, termogravimetria, hmotnostný úbytok.

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Declaration

I declare that the master thesis has been worked out by myself and that all the quotations from the used literary sources are accurate and complete. The content of the diploma thesis is the property of the Faculty of Chemistry of Brno University of Technology, and all commercial uses are allowed only if approved by both the supervisor and the dean of the Faculty of Chemistry, BUT.

Brno, May 8, 2023 ----------

Veronika Stanislavová

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1 INTRODUCTION

In recent years, there has been a growing interest in developing and using bioplastics as an alternative to conventional petroleum-based plastics owing to their chemical and physical properties (Wei et al., 2011). Bioplastics are polymers derived from renewable resources designed to degrade more quickly and efficiently in the environment than conventional plastics (Niaounakis, 2015). Nonetheless, there is still limited knowledge regarding the fate and behaviour of bioplastics in varying environments, especially in the soil ecosystem. Soil is a complex and dynamic environment critical in nutrient cycling and maintaining ecosystem health and quality (Sarkar et al., 2021). The application of bioplastics in agriculture and horticulture, such as mulching films, has increased in recent years (Hayes et al., 2012). Therefore, understanding the biodegradation of bioplastics in the soil is crucial for assessing their environmental impact and ensuring their sustainability as a viable alternative to conventional plastics. The commonly used biodegradable polymers in the agriculture are polyhydroxyalkanoates (PHA). These materials possess various features that enhance their environmental compatibility, including biodegradability, thermoprocessibility, and elasticity (Kadouri et al., 2005; Philip et al., 2007).

Due to the lack of studies examining the effects of bioplastics on soil properties and biota, the ongoing research primarily examines the effects on the terrestrial ecosystem using the findings from analysis of conventional plastics (Liwarska-Bizukojc, 2021). As the use of bioplastics increases, it is vital to understand their interactions with soil ecosystems and identify research gaps. The objective of this thesis is to examine the degradation of bioplastics in soil, with a particular emphasis on the factors that affect their degradation, biodegradation mechanisms and pathways, and the impacts of biodegradation on microbial communities and soil properties. The research methodology involves laboratory assays and techniques to determine the influence of the biodegradable biopolymer poly-3-hydroxybutyrate (P3HB) on soil properties. P3HB is added to the soil alone or in combination with other organic amendments (compost, biochar, manure and separate) to assess their potential impact. This study is based on the premise that the biodegradation of P3HB negatively influences plant growth, but this effect can be improved by incorporating organic amendments prior to the application of P3HB. Furthermore, it is assumed that the biodegradation rate of P3HB could be further supported by applying P3HB after the initial application of amendments.

2 THEORETICAL PART

2.1 Bioplastics

Bioplastics are a blend of polymers with organic and inorganic additives. They comprise petrochemical constituents derived from coal, oil, and natural gas. Due to their broad spectrum of applications in industrial and domestic use, plastics have gradually become the most prevalent material on the market. However, most fossil-based and bio-based plastic materials are non-biodegradable and can persist in the environment for hundreds of years (Ahmed et al., 2018; Nanda & Berruti, 2021; Shah et al., 2008). This can alter ecosystems' properties and pose a threat to biota through ingestion and bioaccumulation, which can lead to health hazards. Plastic waste disposal is a growing concern due to its limited recyclability, leading to accumulation in landfills, soil and the endangerment of water contamination and marine life from plastic debris in oceans (Ahmed et al., 2018; Nanda et al., 2022; Nanda & Berruti, 2021; Shah et al., 2008). In addition, plastics have been known to release harmful chemicals, such as bisphenol A (BPA) and phthalates, which can leach into food and water supplies and cause health problems. Furthermore, plastic production and disposal demand a substantial amount of energy and contribute to greenhouse gas emissions and climate change (Corrales et al., 2015; Narancic et al., 2020).

As the negative impacts of synthetically produced plastic material on the environment and human health become increasingly evident, there is growing interest in biologically produced alternative polymers (Ahmed et al., 2018). Bioplastics are derived from natural renewable sources such as polysaccharides (e.g. cellulose, starch, or chitin), lipids (oils), proteins (e.g. gelatin and gluten), plant/microbial polyesters (polyhydroxyalkanoates) or synthesized from renewable sources (e.g. polylactic acid from starch). These biopolymers could reduce the production of, and eventually replace, conventional petroleum-based plastics, thereby reducing carbon dioxide (CO_2) emissions associated with their production or combustion. Biopolymers differ from petroleum-based polymers primarily because of their sustainability and biodegradability (Ashter, 2016a; Flieger et al., 2003). Based on their biodegradability, bioplastics can be classified into two main categories, non-biodegradable and biodegradable. This categorization is illustrated in Fig. 1.

Non-biodegradable bioplastics, which are typically made from renewable resources, can be classified as either bio-based or fossil-based polymers. Although these bioplastics contain biobased components, they are chemically identical to their petrochemical counterparts. Some of the commonly used non-biodegradable bioplastics include bio-based polyethylene (PE), bio-based polyethylene terephthalate (PET), and bio-based polyamide (PA). However, most currently used conventional plastics are either non-biodegradable or degrade at such a slow rate that total disintegration is impossible (Ahmed et al., 2018; Andreeßen & Steinbüchel, 2019; Ghosh et al., 2013).

Biodegradable bioplastics are classified as either bio-based or fossil-based polymers depending on their biodegradability (Ahmed et al., 2018). The European Norm EN 13432 defines the term “biodegradable” as the type of material that undergoes degradation by the breakdown of organic chemicals by microorganisms (MO) under oxic conditions (in the presence of oxygen) or anoxic conditions (in the absence of oxygen) (European Committee for

Standardisation, 2000). The effectiveness of the biodegradation process is influenced by microorganism type, pre-treatment nature, and polymer properties, such as mobility, crystallinity, functional group type, tactility, chemical components, molecular weight, and additives included in polymers (Artham & Doble, 2008). Moreover, degradation is influenced by abiotic factors such as temperature, moisture salinity, UV radiation or chemical conditions (Andrady, 2011).

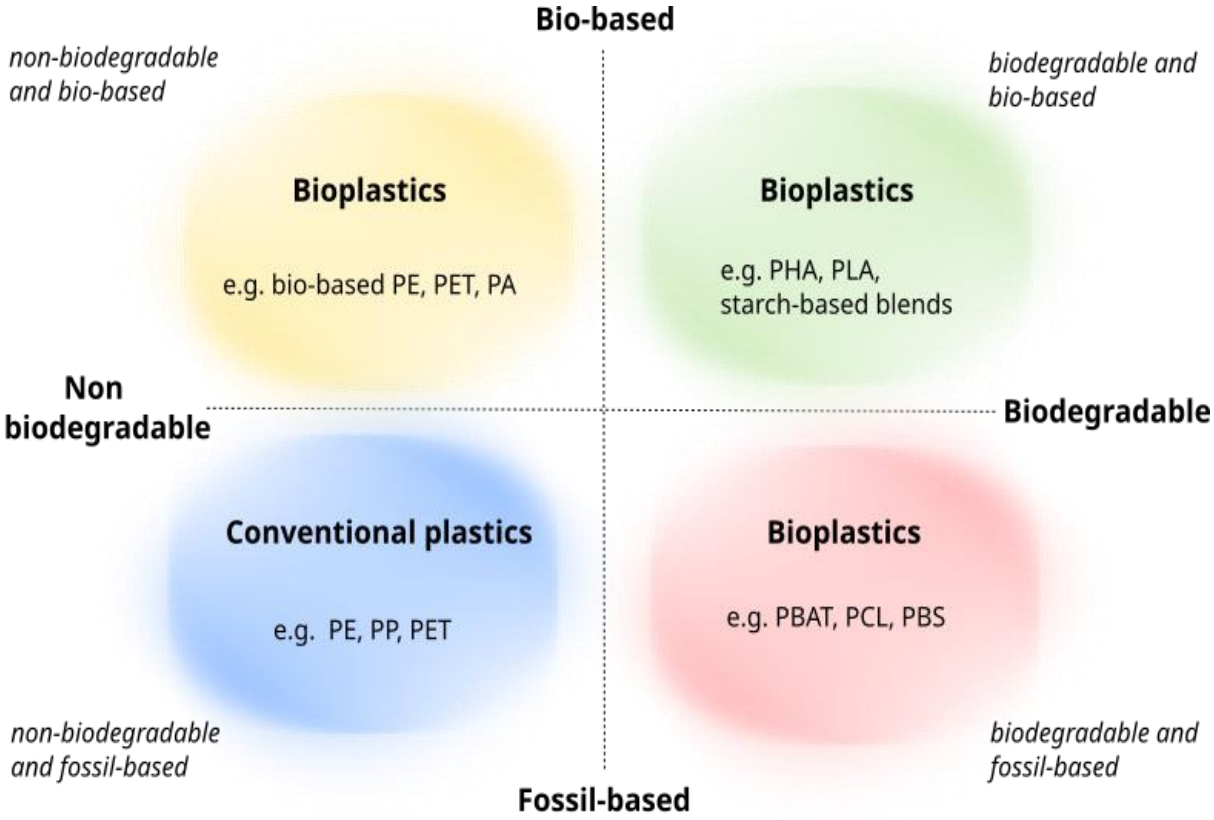

Fig. 1: Classification of bioplastics (Shah et al., 2021).

Biodegradable polymers can disintegrate into smaller molecules and eventually break down into natural substances through biological processes such as oxidation or hydrolysis (Ashter, 2016b). As biodegradable polymers degrade, their constituent parts are separated and reintroduced into nutrient cycles, such as carbon (C), nitrogen (N), and sulphur (S), water (H_2O), CO_2 and biomass are produced under aerobic conditions, whereas hydrocarbons, methane, and biomass are produced under anaerobic conditions (Lucas et al., 2008; Shah et al., 2008). Biodegradable polymers can be further classified into two main categories:

1. Natural biodegradable polymers derived from biomass or renewable resources, such as plant and animal materials (e.g. cellulose, starch, and proteins), can biodegrade through natural processes (Ashter, 2016b).
2. Synthetic biodegradable polymers are either synthesized from fossil fuel-based resources or renewable feedstock (e.g. polylactic acid, PLA; polycaprolactone, PCL) (Ashter, 2016b).

Although bioplastics account for only 1% of total plastic production, there is a growing trend towards their wider use. According to data provided by European Bioplastics in 2022, biodegradable plastics (PLA, PHA, starch blends) account for over 51% (1.1 million tonnes) of global bioplastics production. This is expected to reach over 3.5 million tonnes by 2027. Non-biodegradable biobased plastics such as bio-based PE, bio-based PET and bio-based PA account for 48%, with their relative share predicted to decrease (see Fig. 2) gradually (PlasticsEurope, 2022).

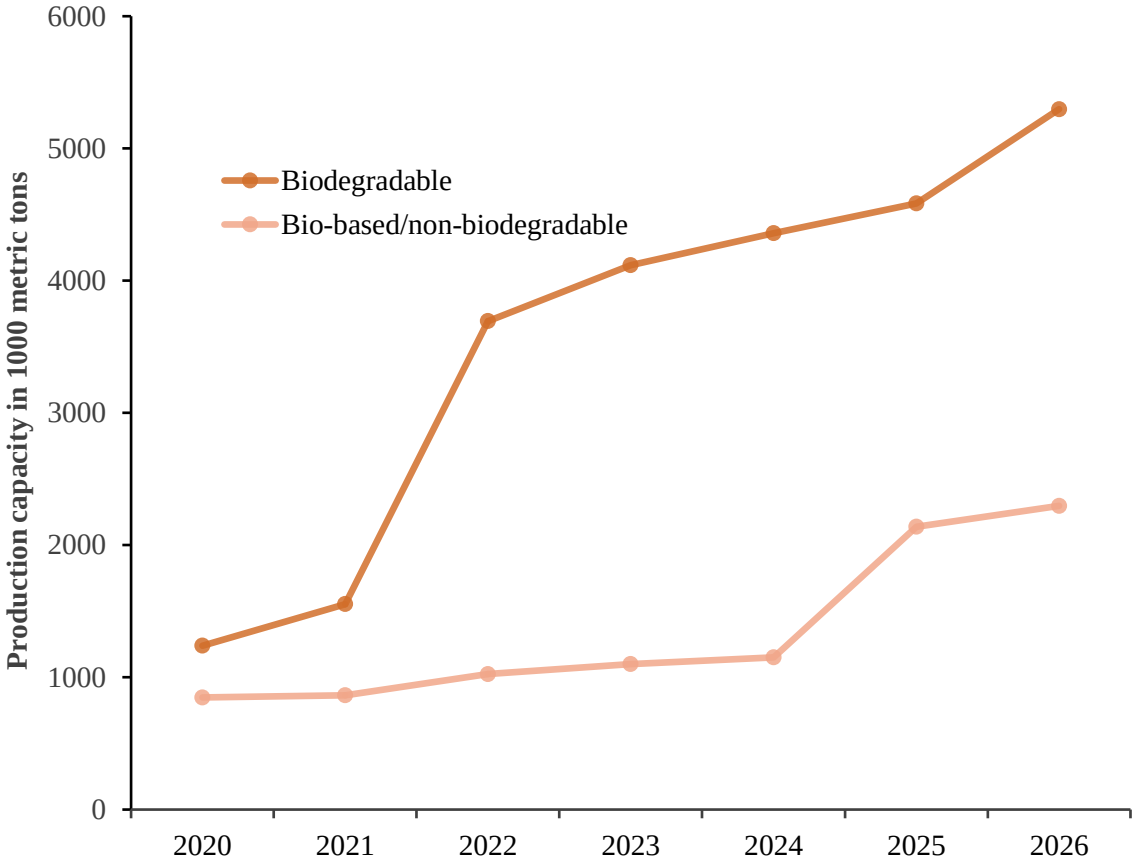

Fig. 2: Bioplastic production capacity worldwide (European Bioplastics, 2021a).

2.1.1 Application of bioplastics.

Biopolymers have attracted significant interest in recent years because of their characteristic properties and ability to address various challenges in various fields. Bioplastics help to enhance the performance of other biologically active molecules in a product because of their biocompatibility and biodegradability. Furthermore, they can be altered to meet potential applications such as medical devices, food packaging, agriculture/horticulture, textiles, or cosmetics (Ashter, 2016a). Even though the packaging is still the largest market for bioplastics, comprising almost 48% (1.2 million tonnes) of the total market in 2021, functional polymers are expanding into new sectors such as automotive, transport, and construction with increasing capacities (see Fig. 3). In this section, the diverse applications of biopolymers, including their use in the packaging, biomedical, agricultural, textile and environmental sectors, will be discussed.

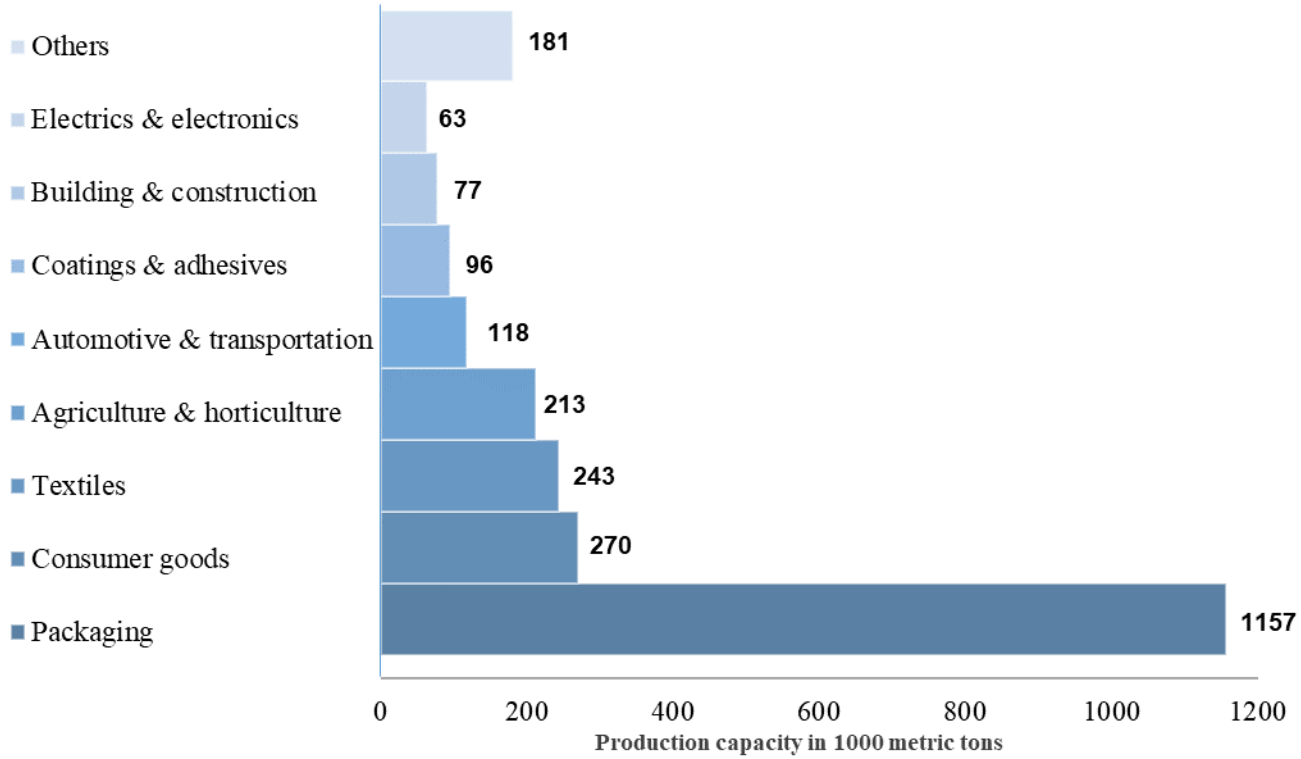

Fig. 3: Global bioplastic production capacity by market segment 2021 (European Bioplastics, 2021).

2.1.2 Applications in medicine

During the past half century, biodegradable polymers have been a central focus of biomedical research. Significant progress has been achieved in their use as controlled drug-release vehicles and in the production of therapeutic devices, including implants and three-dimensional scaffolds for tissue engineering applications (Narancic et al., 2020). Biomaterials made of proteins, polysaccharides, and synthetic biopolymers are commonly used in medical applications, but their mechanical characteristics and stability in aquatic environments are limited. Cross-linking can enhance these properties, but most cross-linkers adversely affect biopolymer function or are toxic to cells. Glutaraldehyde is a popular cross-linking chemical, but its cytotoxic effects in cross-linked biomaterials are debated (Mohan et al., 2016; Othman, 2014).

Gelatine is a versatile biopolymer widely used in medical applications due to its self-assembling properties, non-toxicity, biodegradability, cost-effectiveness, and non-immunogenicity. Techniques such as porogen leaching or gas foaming can create porous gelatine scaffolds and films for drug or nutrient delivery to aid the healing process in wounds (Mohan et al., 2016; Van Vlierberghe et al., 2007).

To be effective as drug delivery systems (DDS), biodegradable polymers should self-assemble into nanocarriers that can load specific drugs, ensure safety, achieve maximum drug encapsulation, maintain bioavailability, biodegrade compatibly with tissue healing, avoid toxicity and immune response, and have specific physicochemical characteristics such as composition and charge (B. Liu & Thayumanavan, 2017; Narancic et al., 2020). Nanocarrier

success depends on physio-chemical features like composition, charge, stability, and size distribution. Effective DDS uses amphiphilicity and hydrophobicity for targeted delivery (Narancic et al., 2020; Palanikumar et al., 2020). Polyester nanoparticle DDS, including PHB-produced polymer-drug conjugates and amphiphilic block copolymers, are promising materials (Barouti et al., 2017).

Scaffolds for tissue engineering must resemble the structure and mechanical properties of the replaced tissue (Lomas et al., 2013). Both natural and synthetic polymers have been studied for this purpose (Fan et al., 2009). Biopolymers are useful in tissue engineering due to their potential to replace immunogenic biogenic materials. Hydrophobicity, biocompatibility, and nontoxicity are essential factors for biopolymers in this field (Falde et al., 2016; Morris et al., 2017; Narancic et al., 2020). Common biopolymers used in tissue engineering include PHA, PLA, poly(lactic-co-glycolic acid) (PLGA), polybutylene succinate (PBS), and poly-γ-glutamate. PLA and PLGA are particularly interisting due to their melting and glass transition temperatures (Narancic et al., 2020). PHBHHx, a random copolymer of 3-hydroxybutyrate and 3-hydroxyhexanoate, has the potential for tissue engineering due to its adaptable mechanical properties, biodegradability, and compatibility with various types of mesenchymal cells. However, its breakdown can generate acidic residues that could affect the physiology of cells or target tissue (Lomas et al., 2013; Narancic et al., 2020).

2.1.3 Application in the food industry

Food packaging has three fundamental functions: food containment, quality preservation and protection against various environmental, physical, and microbiological factors (Han, 2014). Biopolymers have multiple uses in the food industry, including packaging, coatings, and encapsulation (Mohan et al., 2016).

Packaging materials made from petroleum-based polymers are extensively used due to their moldability, affordability, printability, and excellent resistance to various environmental and mechanical factors (Horodytska et al., 2018). Commonly used conventional plastics include polyethylene, polypropylene (PP), polyethylene terephthalate, and polystyrene (PS) (Narancic et al., 2020). Nevertheless, the use of plastic packaging has negative consequences on the environment, as it can take hundreds of years to degrade, ultimately accumulating waste. Additionally, plastic packaging has been linked to releasing chemicals (mainly additives such as bisphenol A and phthalates) that may compromise the quality and safety of food products (Bhargava et al., 2020; Chamas et al., 2020; Hahladakis et al., 2018). The food industry is the largest consumer of packaging materials. Even a minor reduction in the number of materials needed for each package can result in cost reductions and address concerns about solid waste (Han, 2014).

In recent years, the food packaging industry has prioritized sustainability due to consumer, manufacturer, converter, and retailer concerns. The utilization of biobased polymers in food packaging applications is gradually increasing (Narancic et al., 2020). Bioplastics may reduce greenhouse gas emissions and landfill waste, but many require industrial composting facilities for biodegradation, which are not widely available due to insufficient regulations in most countries (Byun & Kim, 2014; Yu & Chen, 2008). Biodegradable polyesters and thermoplastics like PHA, starch, and PLA are currently the most economically feasible materials for food packaging and are already used in several applications (Mohan et al., 2016). PHA polymers are

promising alternatives to fossil-based plastics such as low-density polyethylene (LDPE) and polypropylene (PP) due to their comparable barrier and mechanical qualities (Ray & Kalia, 2017).

When selecting biopolymers for food packaging, barrier qualities are crucial, and hydrophilic polymers are not ideal due to their low moisture resistance. However, the addition of nanofillers, such as nano clays and metal oxide nanoparticles, to biopolymers can enhance the barrier characteristics. Other biopolymers, such as proteins (e.g., zein), polysaccharides (e.g. chitosan), and lipids s (e.g., waxes), have potential as gas and aroma barriers but have limitations such as rigidity and difficulty in processing (Mohan et al., 2016). Polyglycolic acid (PGA) is a promising biopolymer with excellent barrier characteristics (Mohan et al., 2016; Samantaray et al., 2020). Other bio-based biopolymers such as bio-PE, bio-PP, and bio-PET have similar properties to oil-based plastics and can be recycled. Still, they are not biodegradable and have low recycling rates (Narancic et al., 2020).

Biopolymers such as chitosan, lysozyme, and amylose are used for edible encapsulation and coatings to enhance foods' safety and shelf life by incorporating functional ingredients. However, their hydrophilic nature can impact barrier properties and require plasticizers to modify mechanical properties, which requires the careful selection to prevent anti-plasticization (Mohan et al., 2016).

Nevertheless, compared to synthetic thermoplastic polymers, biopolymers face challenges such as high costs, processing difficulties, and inferior functional and structural properties (Mensitieri et al., 2011). The limitations of commonly used biodegradable plastics, including PLA, PHA, PCL, starch, and cellulose, are characterized by various factors such as brittleness, low melt strength, thermal instability, poor barrier properties, mechanical stiffness, poor hydrophilic properties, difficulty in processing, poor stabilization, and low water vapour barrier (Cyras et al., 2007; Din et al., 2020; Jabeen et al., 2015; Rhim et al., 2009). However, techniques such as coating, blending, and modification can enhance their ability to block gases and water. For example, polylactic acid/thermoplastic starch blends show promise in food packaging due to their superior long-term stability compared to other biodegradable plastics (Din et al., 2020).

In conclusion, biopolymers offer a promising solution for reducing the environmental impact of food packaging while providing functional benefits. However, transitioning to biodegradable packaging should not excuse unsustainable practices like excessive plastic consumption and disposal habits. Despite some challenges, the versatility of biopolymers in food-related applications continues to expand, and their potential in the food industry looks promising (Narancic et al., 2020).

2.1.4 Application in the textile industry

The textile industry is a significant contributor to environmental pollution, despite offering a wide range of products such as traditional clothing, filtering media, and protective textiles. Textile fibres can be natural or synthetic; conventional production of natural fibres can be unsustainable due to the excessive use of water, pesticides, and animal cruelty (Nayak et al., 2023). Currently, most textiles today are made from non-biodegradable, petroleum-based synthetic fibres like polyester, nylon, acrylic or spandex (Karthik & Rathinamoorthy, 2018). Synthetic fibres are cheap and have better qualities than bio-based fibre, but their production requires non-renewable resources, harmful chemicals and large amounts of energy, leading to

air and water pollution, greenhouse gas emissions, and other negative environmental impacts (Jahandideh et al., 2021; Nayak et al., 2023).

However, with the depletion of oil sources and the implementation of environmental regulations, biopolymers have emerged as a sustainable and ecologically sound alternative for the textile industry to preserve the environment (Jahandideh et al., 2021; Nayak et al., 2023). For instance, biodegradable polymers such as PLA, PCL, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and poly(trimethylene terephthalate) are gaining popularity in the textile industry due to their biodegradability, renewability, and potential for sustainable production (Jahandideh et al., 2021; Nayak et al., 2023).

Biopolymers have already been used for various applications in the textile industry, such as dyeing, printing, finishing, and production of technical and functional textiles. They can be used as raw materials for woven or nonwoven textiles, binding agents (e.g. chitosan), viscosity modifiers (e.g. alginate), and thickener agents (e.g. carrageenan). Biopolymers can also be blended with other fibres to create unique textile characteristics (Jahandideh et al., 2021; Nayak et al., 2023). For example, PLA can be combined with cotton or bamboo fibres to improve mechanical properties (Tokoro et al., 2008), and chitosan can be blended with other fibres to produce antimicrobial fabrics (Hosseini et al., 2009). The textile industry is moving toward biodegradable polymers to meet the demand for sustainable products and reduce dependence on petroleum. Despite potential drawbacks such as weaker mechanical properties and higher susceptibility to heat, this trend is expected to continue despite technological challenges (Jahandideh et al., 2021).

Water purification

Water treatment and purification play a critical role in ensuring access to clean and safe water, which is essential for human health and environmental sustainability. The use of biopolymers, mainly those derived from natural sources, is being increasingly explored as a sustainable and effective solution in this area. Many have shown superior performance in removing heavy metals and organic contaminants from water compared to conventional synthetic polymers (Mohan et al., 2016; Udayakumar et al., 2021).

Chitosan, derived from crustacean shells, is a biodegradable flocculant that can remove metals and organic molecules (e.g. dyes, pesticides, drugs, endocrine disruptors) from water. Although developing stable materials is challenging, chitosan is still considered a safer alternative to synthetic flocculants. Its chelation capacity and biodegradability (within weeks or months) make it suitable for treating challenging industrial stormwater and wastewater where other approaches have failed to reduce contaminant levels (Mohan et al., 2016; Udayakumar et al., 2021; Desbrières & Guibal, 2018).

Another example of a biopolymer used in water treatment is alginate, which effectively removes metallic ions from water through an ion exchange mechanism. However, modifiers are combined with alginates to enhance their ability to remove heavy metals and dyes due to their poor elasticity and mechanical properties (Wang et al., 2019). Cellulose, another biopolymer, is an inexpensive adsorbent that can be chemically modified to increase its capacity for adsorbing heavy metals and dyes (O'Connell et al., 2008). Porous graphene oxide biopolymer

gels also effectively eliminate heavy metal ions and cationic dyes from wastewater (Cheng et al., 2013).

2.1.5 Application in agriculture

Biodegradable polymers are increasingly used in agricultural coverings such as mulch films, sowing bands, pots, containers, and other horticultural materials and tools. Furthermore, they are used for the controlled release of agricultural chemicals and fertilizers or other applications, including fishing lines, traps, artificial baits, cures, and aquaculture (Niaounakis, 2015). Biodegradable polymers used for applications in this field are primarily PLA, PHA, and starch-based materials (Amelia et al., 2019). In addition, bioplastics can be used in other agricultural applications. For the purposes of this thesis, only the most common applications will be discussed in more detail.

Mulch films

One of the most popular applications of plastics is agricultural mulching due to its ability to provide benefits such as crop protection, increased yield, preservation of soil structure, moisture retention, temperature regulation, or weed and pest control (Niaounakis, 2015). There are two types of mulch in agriculture: synthetic and natural. Synthetic mulch is more effective for weed control and water retention, making it more popular for large-scale agriculture. Although synthetic mulch is more expensive than organic mulch, it is preferred due to its effectiveness in suppressing weeds and conserving water (Barnes et al., 2009; Kasirajan & Ngouajio, 2012; Somanathan et al., 2022).

Plastic mulches may be applied for years on the soil (e,g, strawberries, asparagus) and eventually break down into microplastics. These microplastic residues can spread throughout the soil ecosystem and cause negative effects on physiochemical soil properties, pH, soil structure, water retention, nutrient cycling, plant growth, and ecosystem productivity (Gao et al., 2019; Somanathan et al., 2022; Wang et al., 2020). They can even affect soil fauna and soil microbes. Furthermore, microplastics can also act as vectors for pollutants and heavy metals, resulting in their accumulation in soil and potentially negative environmental and human health consequences (Hartmann et al., 2017). The removal of the film and the irrigation system is a complex and costly process, and the removal of the contaminated film can be problematic due to the presence of plant matter, dirt, and potentially harmful substances such as pesticides and fertilisers (Brodhagen et al., 2015; Niaounakis, 2015; Somanathan et al., 2022). Various methods are available for the disposal of mulch films, including burning, incineration, recycling, composting, and landfilling. However, each has significant economic or environmental drawbacks (Hayes et al., 2012; Ren, 2003).

Bio-based biodegradable polymers, including PHA, PLA, starch, cellulose, polybutylene succinate, and ethylene vinyl acetate, as well as fossil-based polyesters like poly(butylene succinate-co-butylene adipate) (PBSA), polybutylene adipate terephthalate (PBAT) and PBS, have been extensively used to develop biodegradable films that can meet several requirements, such as air and moisture permeability, weed prevention, and light-blocking properties (Kasirajan & Ngouajio, 2012; Niaounakis, 2015). These films can be an alternative to conventional PE films, providing similar functions in soil moisture preservation, temperature

regulation, soil structure and fertility enhancement, weed control and soil salinity management (Jia et al., 2020).

However, they also improve soil physical characteristics, reduce tillage and irrigation needs, and limit agrochemical use, making them ideal for speciality crop production. This can lead to a more sustainable agroecosystem by increasing water-air ratios and temperature conditions in the soil and providing a better habitat for microbial organisms (Abbate et al., 2023). Biodegradable mulch films weaken with time, making them easy to plough into the soil for complete decomposition. Their strength can be controlled by reducing their resistance to natural conditions like temperature, humidity, and light. Using biodegradable mulch films can save labour and disposal costs by allowing incorporation through soil tillage operations, preserving resources, and reducing pollution (Kasirajan & Ngouajio, 2012; Niaounakis, 2015). However, due to the poor mechanical properties of starch, which is often used to manufacture these films, it must be mixed with other polymers and plasticizers (Niaounakis, 2015).

So far, the use of biodegradable mulch films is not without drawbacks. In reality, its complete breakdown in the soil is not always guaranteed, raising concerns about its impact on soil ecosystems (Bandopadhyay et al., 2018; Goldberger et al., 2015). Biodegradable mulch films are more expensive than conventional mulches, but they save on labour and disposal costs. They have a lower tensile strength and a thinner nature, requiring extra care during handling to avoid tearing. The mechanical properties of the film deteriorate significantly after six months from production. Furthermore, they may begin to deteriorate or fuse during transportation or storage, and the weight of the film can cause it to adhere to itself. Weather fluctuations can affect their degradation, making developing a material that functions consistently across different regions and over time difficult (Hayes et al., 2012; Li et al., 2014; Niaounakis, 2015). As a result, adoption rates among farmers are low. However, multiple patent applications have addressed the abovementioned complications and improved the utility of biodegradable mulch films (Niaounakis, 2015).

Moreover, gellan gum and gaur gum with thermoregulation properties can enhance the strength, durability, and resilience of sandy and clayey soil when used as **soil additives** in agriculture (Fatehi et al., 2018). Therefore, selecting appropriate biopolymers is crucial for improving soil quality in agricultural applications (Niaounakis, 2015).

Biopolymers like PHA (primarily P(3HB-3HV) and P3HB) and PLA have agricultural uses for **pest control and enhancing plant nitrogen fixation**. Biopolymer pellets containing insecticides are sown with crops to control pests, with the degradation of the polymer controlling the rate of insecticide release (Holmes, 1985; Philip et al., 2007). Biopolymers are also used as carriers for bacterial inoculants to enhance nitrogen fixation in plants to increase crop yield. These inoculants must withstand stressful environmental conditions (Kadouri et al., 2005; Philip et al., 2007). PHA-rich *Azospirillum* cells in peat inoculants significantly increase crop yield, improving commercial inoculants' shelf life, efficiency, and reliability, impacting agricultural sustainability field experiments with maize and wheat (Dobbelaere et al., 2001; Kadouri et al., 2005). The drawbacks of using biopolymers for these purposes are comparable to those mentioned earlier, including high production cost, limited availability, degradation, efficiency and regulatory approval requirement (Kadouri et al., 2005; Philip et al., 2007).

Fertilizers and grow bags are used in agriculture for seedling development, providing soil temperature stabilization, moisture retention, and crop protection. They also improve crop survival rates and facilitate transportation. However, standard plastic bags pose environmental and agricultural problems, so there is a need for biodegradable alternatives. PHA agricultural grow bags are a beneficial alternative since they are biodegradable, do not leave toxic residue in the soil, and can remove nitrogen from water without contamination (Amelia et al., 2019; Hiraishi & Khan, 2003). Additionally, they promote root growth and prevent root deformation, which can adversely affect plant development, immunity to pathogens, and the ability of the plant to anchor firmly in the soil after transplantation (Bilck et al., 2014). PHA grow bags eliminate double handling and bag recycling after usage (El-Malek et al., 2020). However, they may not be suitable for all crops or growth conditions (Amelia et al., 2019).

Agricultural nets are extensively used to protect crops and improve their quality and yield (Scarascia-Mugnozza et al., 2012). Non-biodegradable plastics are the preferred material for these nets due to their easy and scalable production. However, biobased plastic nets made from polyamine acids, polysaccharide derivatives, PHB, PCL, and PLA have excellent biodegradability compared to conventional plastics like HDPE, PE, and PVC (El-Malek et al., 2020; Maraveas, 2020a). However, bio-based polymers have low tensile strength, and the disturbance of natural insect predators like spiders hinders their use in agriculture. Chemical additions can enhance strength but at the expense of sustainability. Electrospinning processes can produce plastic nets with elastic properties (Maraveas, 2020a, 2020b).

2.1.6 Benefits and drawbacks of bioplastics

The bioplastics are favourable in terms of energy consumption and greenhouse gas emissions compared to conventional plastics. However, other factors must be considered when comparing these two types of plastics, such as the negative environmental impact of fertilizers and pesticides used in the cultivation of bioplastics, as well as the presence of non-biodegradable copolymers. This can increase energy demand and CO_2 emissions (Gironi & Piemonte, 2011). The carbon footprint of bioplastics depends on whether the carbon captured by the plant during photosynthesis is permanently stored in the plastic. Bioplastics made from biological sources can sequester carbon, but carbon sequestration is reversed if they degrade back into CO_2 and H_2O. However, permanent bioplastics can store carbon indefinitely, even after they are recycled multiple times, potentially leading to a much lower carbon footprint (Arikan & Ozsoy, 2015; Chen, 2014). In addition, bioplastics are often free of harmful chemicals like BPA and emit fewer greenhouse gases without any toxins (Arikan & Ozsoy, 2015; Chen, 2008).

Life cycle assessments comparing the environmental impact of recycling petroleum-based plastics and compostable bioplastics show that recycling has a lesser effect overall. However, there are advantages to using biodegradable products for organic waste and disposable cutlery, which can be directly disposed of with organic waste, saving energy and logistics costs (Gironi & Piemonte, 2011).

The mixing of biodegradable plastics with other polymers to achieve desired properties can be problematic. Blending biodegradable plastic with non-biodegradable results in only the biodegradable components being degraded. Copolymers containing biodegradable and non-

biodegradable monomers can cause even more pollution, so whether they can still be considered biodegradable plastics is questionable (Arikan & Ozsoy, 2015; Iwata, 2015).

Biodegradable polymers offer a solution to the environmental issues arising from conventional polymers' disposal. Theoretically, once biodegradable polymers have met their intended use, they biodegrade in the environment rather than requiring recycling. Therefore, biodegradable polymers reduce plastic accumulation in the background and lower waste production costs (Ashter, 2016a). However, in practice, their biodegradation is often uncertain. Specific conditions are required for them to break down effectively. Even if bioplastics are designed to be biodegradable, they may not completely break down in a landfill if the required temperature, humidity, and pH levels are not met. This makes the direct landfilling of biodegradable bioplastics challenging and involves the development of specific sites for their disposal, which requires space, a controlled environment, regular monitoring, and the introduction of particular microorganisms and nutrients to facilitate their degradation. This additional step adds up to the overall cost of disposal (Nanda et al., 2022).

Despite the potential benefits of biodegradable plastics, there are still challenges that make their use difficult. These include the degradation rate determined by the type and ratio of components in a biopolymer, the dependence on environmental conditions, as well as worse properties compared to conventional plastics that may require the use of additives for improvement (Ashter, 2016a). The biodegradation rate requires a start function to trigger instant degradation after use. The required rate is determined by the intended usage. Optimizing the biodegradation rate of biodegradable plastics depends on moulding techniques and material structure, including factors such as crystallinity, lamellar thickness, and molecular conformation. (Iwata, 2015). Furthermore, degraded bioplastics can harm the environment by creating an acidic soil environment that negatively impacts plant growth and soil quality. Therefore, monitoring the long-term effects of biodegradable products is essential to ensure that they fully decompose (Iwata, 2015).

For bioplastics to be sustainable long-term, society must accept and understand their usage, environmental impact, composting, and recycling. This will shape the bioplastic market, increase raw material supply, and compete with conventional plastics in terms of cost, efficiency, and waste management. Large-scale production would positively impact the global economy while addressing environmental pollution (Nanda et al., 2022).

2.2 Biodegradable polymer – poly-3-hydroxybutyrate

This study was conducted using the biopolymer poly-3-hydroxybutyrate, which is a biodegradable and biocompatible polyester, the most common member of the polyhydroxyalkanoates family (Peña et al., 2014). PHA are a class of biopolymers that are synthesized by bacterial cells and can serve as a source of intracellular carbon and energy storage. Among PHA, P3HB is a type of granule-associated protein that is stored in subcellular structures within bacterial cells called P3HB granules. P3HB granules are coated with phospholipids and granule-associated proteins that play an essential role in their production, degradation, and development. PHA synthases are the primary enzymes responsible for the production of PHA, including P3HB, while PHA depolymerase is the enzyme responsible for their degradation (Pötter & Steinbüchel, 2005).

PHA granules are generated within bacterial cells as a result of the action of PHA synthases, which are the primary enzymes in PHA production (Rehm, 2006). Granules are water-insoluble inclusions that are present in the cytoplasm of bacteria and can account for up to 80% (w/w) of dry cell weight or more (Pötter & Steinbüchel, 2005). Four types of proteins have been identified in the context of PHA synthesis and regulation. These include PHA synthase, PHA depolymerase, 3HB-oligomer hydroxylase, PhaPs (PHA inclusion structural proteins), and PhaR (PHA expression regulator) (Pötter & Steinbüchel, 2005).

PHA synthases are the primary enzymes in PHA production and catalyse the polymerization of hydroxyacyl-coenzyme A (CoA) to PHA and free CoA. These enzymes are stereo-selective and can incorporate secondary monomers into PHA by modifying PH3B biosynthesis genes or by introducing new genes that affect the P3HB biosynthetic pathway, depending on the carbon source provided to the bacteria. On intracellular provision of the substrate, the PHA synthase initiates the catalysis of a high molecular weight PHA molecule. The growing PHA chain bonds to the enzyme, resulting in the change of the soluble enzyme into an amphipathic molecule. This leads to the creation of a PHA granule, with the PHA synthase assumed to be covalently linked to the surface (Madison & Huisman, 1999; Peters et al., 2007; Rehm, 2003).

Poly-3-hydroxybutyrate and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) are the most known types of PHA with properties similar to conventional plastics such as PE and PP. Unlike these two traditional plastics, PHA are biodegradable within a year, while biodegradable within a year (Kookos et al., 2019; Suriyamongkol et al., 2007). PHA are of both scientific and industrial interest; within the industry, PHA can be produced from renewable resources such as sugars and plant oils and can exhibit a wide range of mechanical characteristics, from thermoplastics to elastomers (Hisano et al., 2006). This means that they have a wide range of potential applications in various fields, including packaging, agriculture, medicine, and biotechnology. However, despite their potential benefits, PHA currently have limited market share due to their relatively high production costs. The production cost of PHA is typically 5-10 times higher than that of conventional plastics or other biopolymers, which limits their affordability and accessibility (Costa et al., 2019; Kookos et al., 2019).

P3HB is a linear polyester of D-3-hydroxybutyric acid, which is accumulated in intracellular granules by various bacterial organisms as a response to physiological stress (e.g. oxygen or nitrogen deficiency). P3HB can be produced either in pure or mixed forms of bacteria cultures.

For instance, among the common representatives are *Ralstonia eutrophus* or *Bacillus megaterium*. The isotactic stereo-regular structure of P3HB, with a highly degradable R configuration, makes it a promising biopolymer (Bugnicourt et al., 2016). The linear chain structure of P3HB results in a crystalline PHAe (>60%) linked through amorphous regions to form a semicrystalline polymer (Dalton et al., 2022).

Poly-3-hydroxybutyrate has a high molecular weight, ranging from approximately 50 000 to over a million Daltons, which is dependent on several factors, such as the organism, growth conditions, and extraction technique. Furthermore, the biopolymer has a melting point of 170–180 °C and a glass transition temperature of 5 °C (Biron, 2017). P3HB is not crystalline in nature; however, it can be transformed into a more crystalline form during the extraction process. The degree of crystallinity, glass transition, and microstructure determines P3HB's brittleness, which can worsen if stored for extended periods at room temperature. Additionally, it is free of catalytic residue, so the biopolymer does not contain any catalyst residues or impurities that could affect its properties or degrade over time. P3HB is resistant to hydrolysis, with low permeability for oxygen (O_2), H_2O, and CO_2 and resistance to oxidation but limited chemical resistance. Improving its mechanical properties and stability requires adding lubricants and plasticizers to prevent chain degradation during processing, slowing secondary crystallization, and exploring the impact of high-temperature processing, cooling rates, and additives on crystallization, glass temperatures, and biodegradation behaviour (Ashter, 2016; Chen, 2005).

The physicochemical properties and potential applications of P3HB are influenced by its molecular mass, which plays a crucial role in determining its elasticity and mechanical strength. The composition of the culture medium, pH, temperature, and aeration conditions during polymer production affect the molecular mass of P3HB (Millán et al., 2016; Peña et al., 2014). Additionally, molecular mass affects both the thermoplastic and crystallization properties of P3HB. Low molecular weight P3HB exhibits thermal degradation and brittleness at high temperatures (over 180 °C) (Hong et al., 2013). The relative level of expression of biosynthetic enzymes can be affected by the order of the biosynthetic genes (phbA, phbB and phbC) within the phbB operon and the activity of P3HB synthase (Hiroe et al., 2012), the type of PHA synthase present (Agus et al., 2006), and mutations in the P3HB synthase enzyme all affect the molecular mass (Millán et al., 2016; Zheng et al., 2006).

Due to its biodegradable and biocompatible characteristics, P3HB is considered a potential substitute for petrochemical plastics. Although P3HB (Agus et al., 2006) has mechanical properties similar to conventional plastics such as PP or PE, its high level of crystallinity can result in brittleness and low elongation before breaking (Chen & Wang, 2013; Domínguez-Díaz et al., 2015; Millán et al., 2016). Moreover, P3HB has good barrier properties and is more rigid than conventional plastics (Chen, 2005).

2.2.1 Synthesis of P3HB

This section describes how a type of bacteria accumulates P3HB within its cells. P3HB can be naturally synthesized through bacterial fermentation of lipids and sugars obtained from renewable feedstocks such as sucrose, vegetable oils, and fatty acids. This involves the conversion of organic matter to P3HB, which serves as a means of storing carbon and

energy. These polymers are produced by more than 300 species, mainly bacteria. There are two stages involved in this process: synthesis and degradation (Chanprateep, 2010; Peña et al., 2014).

During the synthesis stage, a bacterium begins by condensing two molecules of acetyl-CoA to create acetoacetyl-CoA. This reaction is catalysed by the 3-keto thiolase enzyme encoded by the phbA gene. The resulting molecule, acetoacetyl-CoA, is then converted to 3-hydroxybutyryl-CoA by the acetoacetyl-CoA reductase enzyme encoded by the phbB gene while using NADPH. Finally, the P3HB synthase enzyme produced by the phbC gene polymerizes hydroxy butyryl-CoA monomers to form P3HB and releases CoA (Rehm, 2003; Stubbe et al., 2005). The P3HB biosynthetic genes may be clustered and arranged in one operon phbCAB in certain species, although the order of these genes varies across species; the genes may be unlinked (Peña et al., 2014; Reddy et al., 2003).

More than 60 PHA synthase genes from eubacteria have been cloned and sequenced, and many more have been revealed in the sequenced bacterial genomes (Steinbüchel & Lütke-Eversloh, 2003). Although many of the P3HB production systems use non-genetically modified bacterial strains, some efforts have been made to increase these polymers' production by genetic manipulation. These efforts include changing the metabolism and regulatory systems to favour P3HB synthesis and recombinant gene expression (Peña et al., 2014).

However, the biosynthetic pathways for P3HB compete with central metabolic pathways, such as the tricarboxylic acid (TCA) cycle, fatty acid degradation (ß-oxidation), and fatty acid biosynthesis for precursors. As a result, it can be advantageous to employ recombinant strains in combination with a multistage fermentation process and low-cost raw materials to optimize P3HB production at the industrial level (Peña et al., 2014).

The bacterium utilizes the P3HB reserves for energy production after depleting the external carbon source. In the degradation stage, polymer chains are converted into hydroxybutyrate or P3HB oligomers by an enzyme P3HB depolymerase (produced by the phbZ gene) (Millán et al., 2016; Peña et al., 2014). Subsequently, hydroxybutyrate is oxidized by an enzyme, hydroxybutyrate dehydrogenase, which is dependent on NAD^+, producing acetoacetate. Ultimately, the succinyl-CoA acetoacetate transferase enzyme converts acetoacetate to acetoacetyl-CoA (Millán et al., 2016; B. Rehm, 2007). The synthesis and degradation stages occur concurrently, and the ratio between the two PHAes can affect the molecular mass of P3HB that accumulates within the bacterium (Millán et al., 2016; Ren et al., 2009).

Several bacterial and archaeal species have been identified as producers of PHA. The most effective ones for producing P3HB on a pilot or large scale are *Cupriavidus necator*, *Azohydromonas lata*, and recombinant *Escherichia coli* (Peña et al., 2014). Additionally, members of *Haloarchaea* have been identified as PHA producers. They offer significant advantages due to their ability to grow in hypersaline conditions, utilize low-cost carbon sources, and lyse in distilled water, simplifying polymer isolation and reducing production costs (Peña et al., 2014; Poli et al., 2011).

2.3 Degradation of bioplastics

In general, degradation refers to any procedure that transforms large and complex substances into smaller units. Various physical or chemical alterations can cause the breakdown of long-chain polymers into their building blocks due to exposure to environmental factors (Ghosh et al., 2013). These factors include heat, light, moisture, chemical conditions, or biological activity, according to which degradation is classified as follows:

- Biodegradation – involves the action of living organisms, typically microbes.
- Photodegradation – occurs when exposed to light, often sunlight.
- Thermooxidative degradation – occurs gradually through oxidative breakdown at moderate temperatures.
- Thermal degradation – involves the breakdown of materials at high temperatures.
- Hydrolysis – the process by which compounds react with water (Andrady, 2011).

The functionality of a polymer can degrade as a result of chemical, physical, or biological reactions that result in the breaking of bonds and subsequent chemical transformations. This deterioration can cause various changes in the material's properties, including mechanical, optical, or electrical characteristics (Shah et al., 2008). When evaluating the impact of plastics, it is essential to consider the possible environmental risks posed by soluble byproducts produced during plastic degradation, along with the leaching of small molecules introduced during product manufacturing (Gewert et al., 2015).

Polymer degradation can be identified in various changes in material properties such as crazing, cracking, erosion, discolouration, PHAe separation, or delamination, which typically involve bond scission, chemical transformation, and the formation of new functional groups (Pospíšil & Nešpůrek, 1997; Shah et al., 2008). The polymer degradation rate is influenced by various factors, such as the type, size, structure, and prevailing environmental conditions to which it is exposed (Jasso-Gastinel et al., 2017).

Biopolymer degradation can occur via abiotic or biotic processes. Abiotic and biotic processes frequently coexist, with abiotic breakdown resulting in smaller molecules that are then mineralized by bacteria (Albertsson & Karlsson, 1990). Abiotic degradation often occurs before biodegradation.

Exposure to environmental conditions (i.e. weather, ageing, and burying) subjected polymeric materials to various abiotic degradation factors, ultimately affecting their biodegradability. Typically, abiotic parameters weaken the polymeric structure, leading to unfavourable alterations (Lucas et al., 2008). However, certain abiotic factors may serve as a synergistic factor or initiate the biodegradation process (Jakubowicz et al., 2006).

2.3.1 Abiotic degradation

Abiotic degradation of plastics in the environment can be classified into two broad categories: (1) physical degradation, including changes in the bulk structure of the material, such as cracking, embrittlement, and flaking; (2) chemical degradation, involving molecular level changes, such as bond cleavage and oxidation of long polymer chains. These reactions form new molecules with considerably shorter chain lengths (Chamas et al., 2020).

Chemical degradation

Chemical degradation can occur in the natural environment under ambient conditions, which refers to the typical temperature and pressure in the surrounding air. Generally, this type of degradation is caused either by hydrolysis (requiring H_2O) or oxidation (requiring O_2). These processes can be accelerated by microbial activity, heat, light, or a combination of these factors (Andrady, 2011; Lucas et al., 2008).

Photodegradation

Photodegradation refers to high-energy radiation when UV rays react with photosensitive materials (Ghosh et al., 2013). The energy that transfers from photons to the molecules can occur through photoionization, luminescence, fluorescence, and thermal radiation (Lucas et al., 2008). Photobiodegradable plastics include PHA, PLA, PCL, PBAT, PBSA, and others (Shah et al., 2008). Photodegradability is connected to their ability to absorb the harmful part of tropospheric solar radiation. This comprises the UV-B terrestrial and UV-A radiation that cause direct photodegradation (photolysis, initiated photooxidation). Through heating, the visible component of sunlight increases the polymeric breakdown. Thermal oxidation is accelerated by infrared light (Pospíšil & Nešpůrek, 1997; Shah et al., 2008). Most plastics absorb UV light, stimulating their electrons by increasing reactivity which leads to oxidation, cleavage, and other degradation processes (Shah et al., 2008).

Thermal degradation

Thermal degradation refers to molecular deterioration caused by overheating. The components of the long-chain backbone of the polymer start to separate by the molecular scission and interact with one another when reaching high temperatures. This leads to changes in physical and optical properties. Thermal degradation typically results in changes to the polymer's molecular weight (and molecular weight distribution). Common property changes include lower ductility and embrittlement, chalking, changes in colour, cracking, and a general loss of the majority of other desirable physical qualities (Shah et al., 2008).

Thermoplastic biopolymers degrade at their melting point as a result of the breaking of chemical bonds. The degree of degradation is influenced by molecular weight and comonomer percentage. The degradation temperatures of the most common biodegradable polymers are following, PLA melts at 159 –178 °C, P(HB/HV) at 137–169 °C, and P3HB at 175 °C (Lucas et al., 2008).

Mechanical degradation

Mechanical degradation can occur as a result of compression, tension, or shear forces. These effects can be caused by a variety of factors, including material limits during installation, ageing, air and water turbulences, snow pressure, or damage from birds. Therefore, thermoplastic polymers (such as low-tunnel films and mulches) can undergo several types of mechanical degradation (Briassoulis, 2004, 2006, 2007). In the context of field conditions, mechanical stresses interact synergistically with other factors, such as temperature, solar radiation, and chemicals, to affect the degradation of materials (Lucas et al., 2008).

2.3.2 Biotic degradation/biodegradation

Microbial metabolism plays a crucial role in biodegradation by breaking down organic compounds into simpler forms that can be utilized for energy and growth. However, the majority of polymers need to be depolymerized into smaller monomers in order to be absorbed and biodegraded by microorganisms since they are often too large, high molecular weight to pass through cellular membranes (Ashter, 2016a; Shah et al., 2008). Therefore, the biodegradation process can typically be divided into four stages, (i) microorganisms attack the polymer surface (biodeterioration); (ii) then the polymer chains are broken down into smaller components. (depolymerization); (iii) these smaller molecules are consumed by microorganisms (bioassimilation); (iv) until the polymer is entirely degraded to natural molecules such as H_2O, CO_2, CH_4 and biomass (mineralization) (Lucas et al., 2008). Although biodegradation is not primarily affected by mechanical factors, they can initiate or accelerate it (Briassoulis, 2005).

Biodeterioration

The initial stage of polymer biodegradation, called biodeterioration, is characterized by the fragmentation of biodegradable polymers into small fractions by combined microorganism activity together with abiotic degradation and enzymatic processes (Gu, 2003). Deterioration modifies the mechanical, physical, and chemical properties of a material on a surface level (Lucas et al., 2008). During this step, MO may secrete adhesives or biosurfactants (Koutny et al., 2009) or could quickly adapt to the polymer environment to efficiently colonise and use the substrate (Tribedi et al., 2015).

Materials may be degraded in several ways depending on the composition of the polymer, environmental factors (temperature, humidity, weather, pollution), and the microorganisms involved (Siracusa, 2019). The microorganisms responsible for biodeterioration belong to various taxonomic groups, including bacteria, protozoa, algae, fungi, and lichens (Wallström et al., 2005).

Microorganisms use extracellular polymers to penetrate materials. Microbial species adhere to a material surface and form biofilms (i.e., structured communities of cells). For significant corrosion or deterioration of the underlying materials to occur, the formation of a biofilm is necessary (Gu, 2003; Lucas et al., 2008). Biofilms can damage synthetic polymeric materials in several ways, including surface coating, leaching of additives and monomers, enzymatic and radical attacks, water accumulation, and pigment excretion (Flemming, 1998). Water causes hydrolysis in various polymers, leading to the production of acids, which erode the surface and sequester cations such as calcium (Ca^{2+}), aluminium (Al^{3+}), silicon (Si^{4+}), iron (Fe^{2+}), manganese (Mn^{2+}) and magnesium (Mg^{2+}), while also serving as carbon sources for microorganisms (Lucas et al., 2008).

Microbial activity can lead to a reduction in the dry weight of polymers and induce physical-chemical alterations, resulting in changes in surface morphologies. Subsequently, the microbe activity leads to the formation of cracks and rough surfaces and chemical bonding structures (Mukherjee et al., 2016; Tareen et al., 2022).

Biofragmentation

Smaller polymer fragments generated by abiotic breakdown can pass through cellular membranes and are biodegraded by cellular enzymes within microbial cells; however, certain microorganisms also secrete extracellular enzymes that can act on specific plastic polymers (Gewert et al., 2015; Shah et al., 2008).

Depolymerization

The microorganisms of the biofilm secrete extracellular and intracellular depolymerases (endo- or exoenzymes) that initiate polymeric chain cleavage. As a result, the polymer chain is depolymerized into oligomers, dimers, or monomers that have significantly lower molecular weights and are of a size that allows them to pass through the bacterial semi-permeable membranes. Subsequently, these smaller monomers can then be utilized by bacteria in the next stage (Ahmed et al., 2018; Shah et al., 2008).

Assimilation

During assimilation, microorganisms absorb smaller molecules produced in depolymerization and are taken up and metabolized into primary and secondary metabolites. Microorganisms use them as a source of energy and nutrients (i.e. carbon, nitrogen, oxygen, phosphorus, and sulphur) for their growth and reproduction. The monomers are transported across the cell membrane and oxidized through catabolic pathways (aerobic respiration, anaerobic respiration, and fermentation), leading to the production of ATP and other cellular components. (Lucas et al., 2008) Microorganisms can assimilate molecules resulting from biodeterioration or biofragmentation, leading to their further degradation and recycling (Siracusa, 2019).

Mineralization

The final stage of biodegradation is the mineralization of polymer monomers. Biodegradation occurs under two different conditions, depending on the presence of oxygen; Aerobic biodegradation (in the presence of O_2) results in the production of carbon dioxide, water and microbial biomass, which is typically facilitated by aerobic microorganisms (Ashter, 2016a; Lucas et al., 2008). The aerobic biodegradation process can be visually represented using an equation:

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{biomass} + \text{mineral salts} + \text{residue(s)} \qquad (1)$$

The second one is anaerobic biodegradation, which produces mainly methane, then carbon dioxide, water, and microbial biomass and is usually carried out by anaerobic microorganism consortia under methanogenic conditions (Ashter, 2016a). An equation that visually illustrates the anaerobic biodegradation process is:

$$C_{polymer} \rightarrow CH_4 + CO_2 + H_2O + C_{biomass} + \text{mineral salts} + \text{residue(s)} \qquad (2)$$

Biodegradation is impacted by both the inherent properties of a polymer (e.g. molecular weight, solubility, crystallinity, or presence of additives) and environmental conditions, such as the availability of oxygen and light, pH, temperature, humidity, microorganism, and enzyme type and concentration. Consequently, a polymer can exhibit varying rates of degradation under different environmental conditions, including water, soil, and physiological states (Agarwal, 2020; Gu, 2003). Nevertheless, the biodegradation of polymer substrates rarely leads to

the complete degradation of the polymer material. This is because a small fraction of the polymer will be integrated into microbial biomass, humus, and other natural products (Gu, 2003). Complete biodegradation or mineralization is achieved when no residual material remains, i.e., when the original product has been completely transformed into gaseous products and salts and all the carbon content has been converted into carbon dioxide (Lucas et al., 2008).

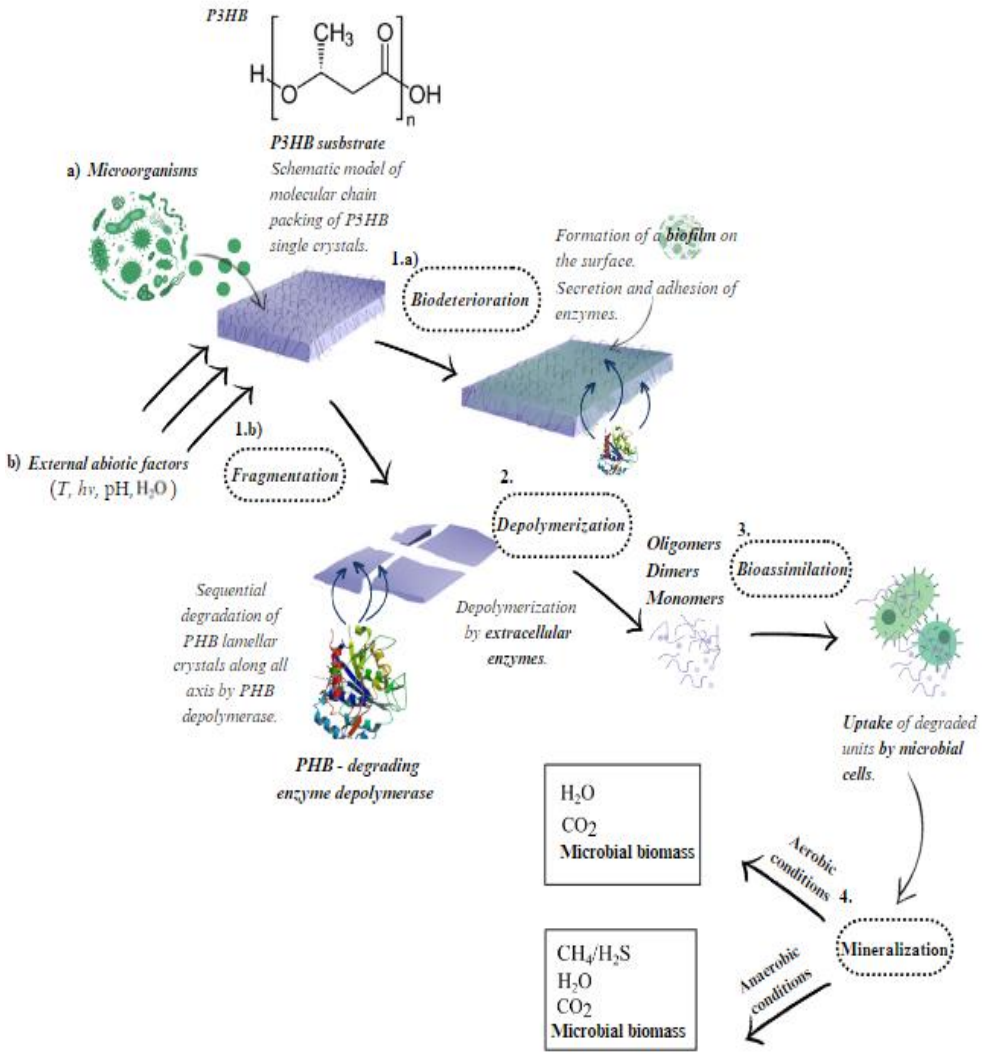

Fig. 4: Mechanism of P3HB biodegradation.

2.3.3 Factors affecting polymer biodegradation

The biodegradability of the polymer is mainly influenced by its specific properties and exposure conditions (Ahmed et al., 2018). The reason for the high resistance of polymers is primarily due to their considerable molecular weight and hydrophobic surfaces, making it challenging for the molecules to penetrate the cell wall (Mohanan et al., 2020). Microbial enzymes can break down low molecular-weight polymers more easily (Auras et al., 2004). The degree of degradability varies depending on the proportion of amorphous and crystalline forms and the presence of strong C-C bonds, which are exceptionally resilient against enzymatic degradation (Mohanan et al., 2020). Enzymes can biodegrade different types of polymers due to their unique active sites (Ahmed et al., 2018). Enzymatic degradability is significantly affected by the polymer's softening temperature; the polymer; with a higher melting point has less possibility

of biodegradation. The potential enzymatic degradation decreases with the increase in temperature (Ahmed et al., 2018; Tokiwa & Calabia, 2004). However, plastics derived from petrochemical sources are generally challenging to degrade due to their hydrophobicity and complex structure (Yamada-Onodera et al., 2001).

Another critical factor in the biodegradation rate is sufficient moisture, which promotes rapid microbial activity and hydrolysis, leading to more chain scission reactions (Ahmed et al., 2018; Ho et al., 1999). Additionally, changes in pH could modify the rate of hydrolysis reactions and microbial growth (Ahmed et al., 2018). The biodegradation rate of polymers is also affected by other factors. The hydrophobic nature of some polymers can hinder the formation of a biofilm of microorganisms, thereby slowing down the biodegradation process (Hadad et al., 2005). Additionally, the shape and size of a polymer are important in the determination of the degradation process. Polymers with a larger surface area tend to degrade faster. Furthermore, there are specific size and shape requirements for the biodegradation of different types of plastics (Ahmed et al., 2018; Kijchavengkul & Auras, 2008). Polymer biodegradability can also be affected by non-polymer substances, such as dyes, catalyst waste, additives, and fillers (Ahmed et al., 2018). However, the addition of biosurfactants can improve the biodegradability of both fossil-based and bio-based polymers, which possess high biodegradability and low toxicity (Hadar & Sivan, 2004). Biosurfactants contain specific functional groups that facilitate the biodegradation process, enabling their activity even under extreme conditions of temperature, pH, and salinity (Kawai et al., 2004).

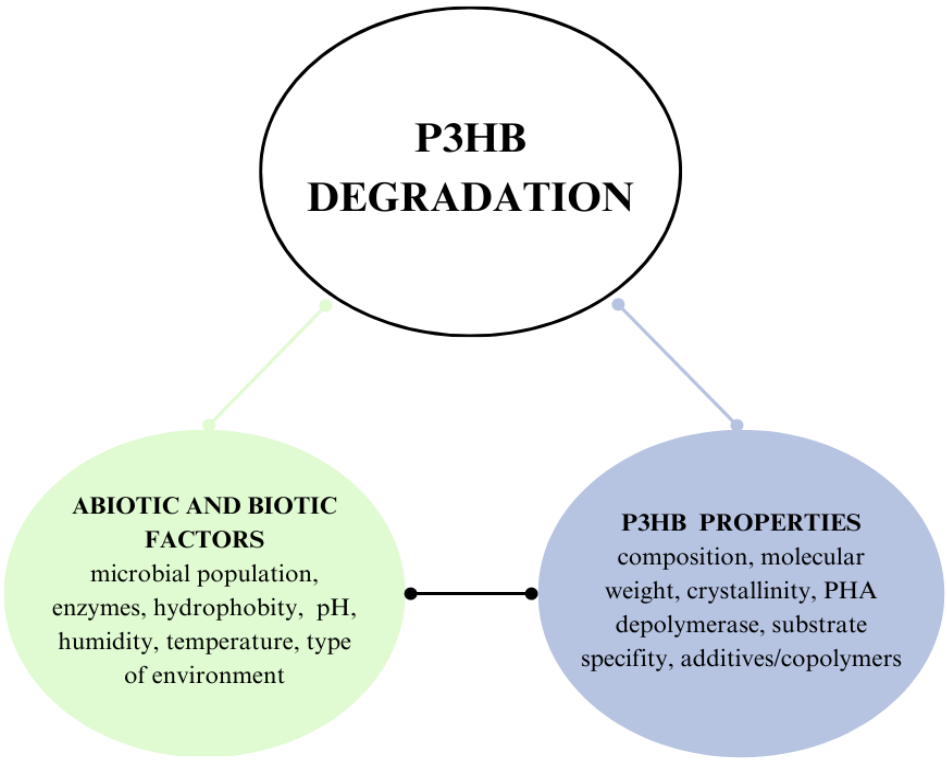

Fig. 5: Influential Factors in the Degradation of PHB (Ong et al., 2017; Tokiwa & Calabia, 2004).

2.4 Soil and its ecosystem function

Soil is the unconsolidated upper part of the earth's crust that acts as a natural medium for the growth of plants. It is a complex and dynamic ecosystem composed of various materials such as minerals, organic matter, water, air, and living organisms (both fauna and flora) (Young et al., 2008). Soil plays a crucial role in food and fibre production and contributes significantly to ecosystem function and environmental quality at local, regional and global scales. The importance of soil as a vital component of the biosphere has led to increasing interest in assessing its quality and health (Doran, 2002).

Soil is a complex natural formation that results from the continuous action of soil-forming processes known as pedogenesis. Soil formation is influenced by the parent material, climate, topography, biota, and time. These factors also affect soil properties, ecosystem function and services (Morgado et al., 2018). The development of soil can be divided into three phases:

- Soil formation – soil-forming agents act on the parent material, gradually developing distinct soil horizons until the soil reaches its typical composition.
- Soil evolution – already-formed soil undergoes gradual changes over time due to natural factors such as weathering, erosion, and biological activity.
- Soil metamorphosis – soil-forming factors, including natural and anthropogenic influences, can cause changes in soil's properties and characteristics (Sarkar et al., 2021).

The soil develops through physicochemical reactions involving parent material. Once the soil is formed, its biogeochemical properties greatly influence its quality. These properties control various chemical reactions, including adsorption-desorption, dissolution, precipitation, and redox reactions, which ultimately contribute to the chemical properties of soil colloids. Understanding soil colloid interactions is crucial for effective soil quality management. Optimizing soil health and fertility promotes sustainable agricultural practices and ensures long-term land productivity (Morgado et al., 2018; Sarkar et al., 2021).

Soil fulfils six essential functions in the overall functioning of ecosystems (Chan et al., 2016; Dazzi & Papa, 2022; Morgado et al., 2018):

(1) Supporting the growth of higher plants by providing nutrients, water, and physical support.
(2) Controlling the fate of water in the hydrological system by regulating the movement and storage of water.
(3) Functioning as nature's recycling system by decomposing and recycling organic matter, nutrients, and minerals, soil regulates several ecological processes through the bio-geo-chemical cycles.
(4) Providing habitat for various biota, including bacteria, archaea, fungi, microalgae, protozoa, nematodes, and bacteriophages.
(5) Serving as an engineering medium or material by providing a foundation for buildings, roads, and other infrastructure.
(6) Influencing the composition and physical condition of the atmosphere by storing and releasing gases such as carbon dioxide, methane, and oxygen.

2.4.1 Soil quality

Soil quality is a fundamental component of environmental quality, along with water and air (Andrews et al., 2002). It refers to the ability of soil to function as a living system, sustain productivity, promote healthy environments, and maintain the well-being of plants, animals, and humans within ecosystem and land use boundaries, according to the definition provided by Doran & Safley (1997). This definition emphasizes the complex and location-dependent nature of soil ecosystems and their connections to ecosystem services. Soil quality is more complicated than air and water quality because it includes solid, liquid, and gaseous PHAes and may provide a broader variety of functions (Bünemann et al., 2018; Nortcliff, 2002).

The terms "soil quality" and "soil health" are sometimes used interchangeably, although they are directly correlated. Soil quality refers to the soil's ability to meet specific human needs, such as crop production. In contrast, soil health encompasses the ecological features of soil that extend beyond its crop production capacity, including its ability to function as a living system and support various life forms (Alkorta et al., 2003).

Soil monitoring involves regular measuring and recording of spatial and temporal changes in soil variables. Detecting any shifts in soil quality at an early stage is crucial. The concept of soil quality goes beyond just soil productivity, as it includes interactions between humans and soil and the sustainability of ecosystems. Nowadays, soil quality assessment is increasingly incorporated into land evaluation for various purposes, such as sustainable land management, environmental risk assessments, ecological change, and land restoration (Bünemann et al., 2018).

Soil quality and health are determined by physical, chemical, and biological properties. These properties can be used as monitoring tools or indicators to evaluate changes in specific soil functions and overall soil ecosystem quality as a result of various management practices and natural disturbances such as climate change and contamination (Doran, 2002; Morgado et al., 2018).

The most proposed soil quality indicators are total organic matter/carbon and pH. These are followed by available phosphorus, indicators of water storage, and bulk density, all of which are mentioned in more than 50% of the reviewed indicator sets. Other frequently used indicators include texture, available potassium (K), and total nitrogen (TN), all used in more than 40% of the reviewed indicator sets (Bünemann et al., 2018).

2.4.2 Indicators used to assess soil quality and health

2.4.3 Physical indicators

Soil Structure (texture, aggregate Stability, porosity)

Soil structure is critical in controlling various soil processes, including water retention and infiltration, gas exchanges, soil organic matter (SOM), mineral nutrient dynamics, root penetration, and erosion susceptibility (Kay, 1990). Additionally, soil structure is a habitat for various organisms, influencing their diversity and regulating their activity. Soil organisms can also actively shape the structure of the soil through their actions, altering the distribution of water and air within their habitats. Therefore, soil structure and soil organisms are closely interconnected and influence each other (Bottinelli et al., 2015; Rabot et al., 2018).

Soil texture, also known as particle size distribution, is a fundamental and constant soil property that affects the soil's physical and chemical properties (Mukhopadhyay, 2020).

Aggregate stability is determined by soil structure and is defined as the resistance of soil aggregates to external energy like heavy rainfall, erosion, or cultivation. It plays a crucial role in several ecosystem functions, including organic carbon accumulation, water infiltration, movement, storage, and root and microbial activity. Measurement of aggregate stability can also help evaluate soil resistance to erosion and management changes (Allen et al., 2011; Dalal & Moloney, 2000).

Porosity refers to the empty spaces in a material and can be measured as a fraction of the total volume. The distribution of pore sizes is vital in determining the capacity of the soil to store water and air, which is essential for plant growth in the root zone (Reynolds et al., 2002). The properties of pores significantly impact the physical quality, affecting bulk density, macroporosity, soil porosity, and water release characteristics. These factors influence several indicators of soil physical quality, such as aeration capacity, plant-accessible water capacity, and relative field capacity (Allen et al., 2011; Reynolds et al., 2009). Additionally, soil porosity and pore size distribution are crucial for root development and soil enzyme activity (Allen et al., 2011).

The water holding capacity is the quantity of water a particular soil can store for crop use. This property is critical for crop productivity. The water retention capacity of soils is typically defined concerning water infiltration, which is the process by which water penetrates the soil and flows its layers to a specific depth (Dalal & Moloney, 2000; Raghavendra et al., 2020). As the infiltration rate varies significantly with soil use, management, and time, it has been included in the assessment of soil health as an indicator of the impact of changes in land use (O'Farrell et al., 2010; Raghavendra et al., 2020).

The bulk density is expressed as the dry soil weight per volume unit. In agricultural systems, it is commonly analysed to describe the state of soil compactness in relation to land use and management (Raghavendra et al., 2020). It also belongs to the valuable indicator of soil health in terms of soil processes such as aeration and infiltration, rooting depth/restrictions, water capacity, soil porosity, plant nutrient availability and soil microorganism activities (Raghavendra et al., 2020; Reynolds et al., 2009). Bulk intensity negatively correlates with the content of SOM or SOC. The loss of organic carbon caused by increased decomposition due to elevated temperatures may increase bulk density. The soil is then more susceptible to compaction through land management activities. Bulk density is a direct measure of compaction and usually does not fluctuate with other soil parameters because it is generally given on a dry soil basis (Raghavendra et al., 2020).

2.4.4 Chemical indicators

Soil pH

One of the most accurate indicators of soil chemical properties is pH. The soil pH is influenced by the solubility of various compounds, the relative bonding of ions to exchange sites, and the multiple microorganisms (Raghavendra et al., 2020).

Electrical conductivity

Soil electrical conductivity is a simple method of determining the salt concentration levels in soil and is widely regarded as a reliable indicator of soil health and quality. It can provide valuable information on various aspects of soil health, such as salinity trends, crop productivity, nutrient cycling (especially nitrate), and biological activity. In combination with pH, it can indicate soil structure, particularly in soils with high sodium levels (sodic soils). (Arnold et al., 2005; Raghavendra et al., 2020). Furthermore, in response to crop management practices, electrical conductivity has been employed as a chemical indicator of soil biological quality (Vargas Gil et al., 2009).

Cation exchange capacity

The cation exchange capacity (CEC) is an essential indicator in determining the chemical quality, as it affects the retention of the major nutrient cations, including Ca^{2+}, Mg^{2+}, and K^{+}, and the immobilisation of potentially toxic cations such as Al and Mn. Therefore, CEC may be used to assess soil health by providing information on the soil's capacity to absorb nutrients, pesticides and chemicals.(Raghavendra et al., 2020; Ross et al., 2008) . Ion exchange capacity primarily influences soil CEC binding to negatively charged organic matter, clay and soil colloid (Raghavendra et al., 2020).

Soil organic matter

Soil organic matter (SOM) encompasses a diverse spectrum of living and non-living constituents. SOM is one of the most complex and heterogeneous soil components, exhibiting substantial variation in its properties, functions, and turnover dynamics (Weil & Magdoff, 2004). It is crucial for soil health and quality, as it contributes to electrical charge, is a reservoir and source of carbon and nitrogen, and affects the cycling of phosphorus and sulfur. Moreover, it provides a habitat for microbes and fauna and influences aggregate stability, water retention and hydraulic properties (Haynes, n.d.; Weil & Magdoff, 2004). Given that SOM plays a fundamental role in driving most soil functions. A decline in SOM content could result in reduced fertility, biodiversity, and a weakened soil structure. The lower content of SOM could further lead to a decrease in water retention capacity, an increase in erosion risk, and bulk density, ultimately leading to soil compaction (Allen et al., 2011; Weil & Magdoff, 2004).

The primary parameter for evaluating SOM conditions is soil organic carbon (SOC), which represents approximately 50% of SOM (Haynes, n.d.; Raghavendra et al., 2020). Soil organic carbon, a fundamental component of SOM, is another essential indicator in assessing soil quality. In general, high levels of SOC levels indicate better soil quality which is generally positively associated with crop yield (Bennett et al., 2010). SOC influences critical functional processes within the soil, such as nutrient storage (particularly nitrogen), water retention capacity, and aggregate stability (Raghavendra et al., 2020). Soil organic carbon variability can result from various factors, such as adding primary organic compounds from photosynthesis distributed unevenly in the soil. The type of soil management practises, such as tilling before crop planting or incorporating amendments and fertilisers, can also cause significant disturbance and stimulate decomposing microorganisms, leading to changes in nutrient concentrations and SOC content (Faria et al., 2009).

Available nutrients

The detection and characterization of both macro- and micronutrients in soil, encompassing elements such nitrogen, phosphorus, potassium, sulfur, iron, and zinc, as well as copper, molybdenum, manganese, boron, and chlorine, can be an instrument to assess soil health. Evaluating soil capacity to provide nutrients to meet the remaining plant nutrient needs beyond the current supply to fulfil the crop requirement (Mukhopadhyay, 2020; Raghavendra et al., 2020). The analysis of extractable nutrients can reveal the soil's capacity for supporting the development of plants; conversely, it may identify critical or threshold levels for environmental risk assessment (Raghavendra et al., 2020).

Priming effect

The priming effect generally relates to short-term alterations in turnover intensity (such as decomposition) of SOM caused by fertilization or plant cultivation (Kuzyakov et al., 2000). It is a process where the addition of organic substrates to the soil can increase microbial activity, which can increase (positive effect) or decrease (negative effect) the rate of decomposition of SOM (Kuzyakov, 2006; Kuzyakov et al., 2000). Its value is determined by the substrate type and SOM quality. Moreover, the positive priming effect contributes to the increasing CO_2 concentration in the atmosphere. (Dilly & Zyakun, 2008).

2.4.5 Soil respiration

Soil respiration is an overall process responsible for the release of CO_2 into the atmosphere, including microbial, above-ground plant, and root respiration. Soil respiration is the result of carbon transfer through the metabolic activity of living organisms, including various microorganisms, including bacteria, fungi, protozoa, and algae, as well as soil fauna (Pell et al., 2006).

Microbial respiration describes the processes by which microorganisms, such as fungi and bacteria, respire and release CO_2 using organic matter as an energy source. It includes all forms of microbial activity, including growth, maintenance, and reproduction; the SOC release rates depend strongly on temperature. It encompasses microbial activity, such as growth, maintenance, and reproduction (Pell et al., 2006). Under controlled laboratory conditions and in the absence of plant roots, a type of microbial respiration called basal respiration reflects the steady rate of CO_2 that microorganisms generate when actively decomposing organic matter (Borken et al., 2002).

Soil respiration is a standard biological indicator of soil health that reflects changes in the health and functioning of the soil environment. It is strongly correlated with the SOM content and the microbial biomass. It is a method of quantifying either the release of CO_2 from the soil or the consumption of O_2. Thus, it can indicate soil respiration rates and microbial activity (Allen et al., 2011). Soil respiration serves as a semiquantitative indicator for the release of nutrients, given that it is affected by changes in nutrient levels and organic matter degradation rates within the soil profile (Borken et al., 2002).

Basal respiration (BR) and substrate-induced respiration (SIR) are well-known and extensively used parameters of microbial activity in soil microbial ecology. Additionally, SIR can be used to determine the total microbial biomass in the soil. Soil microbial biomass carbon is regarded

as the most dynamic and inert component of soil organic carbon. Therefore, the pool of soil microbial carbon, its activity, and composition are critical characteristics in soil processes that have been extensively researched in various ecological approaches (Ananyeva et al., 2008).

Soil basal respiration is the steady rate of respiration in soil originating from the mineralisation of organic matter. It is estimated based either on the release of CO_2 or consumption of O_2. The measurement of soil basal respiration has been applied in various research studies. Both soil microbial respiration and the mineralisation of organic matter are commonly acknowledged as key indicators for measuring changes in soil quality (Creamer et al., 2014; Dilly & Zyakun, 2008). The basal respiration rate is determined by the quantity and quality of the carbon source. As such, BR can serve as an integrated indicator of the potential of the soil biota to decompose both endogenous and exogenous organic substances in response to specific environmental conditions (Pell et al., 2006).

The substrate-induced respiration is a method based on the maximum initial respiratory response of soil microorganisms to the addition of substrate to the soil. It detects bacterial biomass on the principle that excess substrate metabolism is limited by the quantity of active aerobic microorganisms in the soil. The SIR should indicate the impacts of temperature, water availability and resource quality on microbial communities at a given time since optimal circumstances result in growth, whereas periods of stress result in reduced growth and mortality of MO. When provided with standardized conditions, the metabolism of an excess substrate (e.g. glucose) is limited by the number of active aerobic microorganisms in the particular soil (Dilly & Zyakun, 2008; Langer & Rinklebe, 2011).

Soil microbial biomass, a living component of soil organic matter (SOM), refers to the abundance of microorganisms in the soil. These microorganisms regulate nutrient cycling, energy flow, and ecosystem production. Soil microbial biomass values can be used to assess soil nutrient levels and are important in the primary productivity of various biogeochemical processes in terrestrial ecosystems (Gregorich et al., 2000; Raghavendra et al., 2020). Microbial biomass has been proposed as a reliable indicator of soil quality and health due to its sensitivity to changes in soil conditions (Raghavendra et al., 2020).

For the impact on soil functions and the total amount of immobilized carbon, three major characteristics of soil microbial biomass are often considered: pool size, activity, and diversity. Many studies have shown that C and N availability, pH, and moisture content limit activity and growth (Langer & Rinklebe, 2011). Several physiological, biochemical, and chemical methods have been established to measure soil microbial status. These include substrate-induced respiration, chloroform fumigation incubation, and adenosine triphosphate (ATP) analysis (Horwath & Paul, 1994).

2.4.6 Soil enzyme activity

Enzymes play a vital role in the breakdown and conversion of organic molecules into minerals. Monitoring multiple soil enzyme activities is crucial to understanding the biodegradation of organic compounds and the mineralization of carbon, nitrogen, phosphorus, and sulfur. Such monitoring may also reveal the harmful effects of chemicals and other anthropogenic impacts (Alkorta et al., 2003; Rao et al., 2014).

Soil enzymes are crucial indicators of soil quality, as they can reflect changes in the environment of the plant-soil system on a microbiological and biochemical level. It is related to their participation in cycling nutrients, mainly carbon, nitrogen, phosphorus, and sulphur. The processes involved in nutrient cycling involve the complex interplay between biological, chemical, and physical processes within the soil. Enzymes facilitate all biochemical processes within an ecosystem, including the decomposition and synthesis of soil organic matter (SOM). SOM, in turn, positively affects the production of microbial enzymes (Alkorta et al., 2003; Raiesi & Beheshti, 2014; Shi, 2010; Sinsabaugh et al., 2008).

Depending on the location, enzymes can be extracellular or intracellular. Intracellular enzymes are found in cells, whereas extracellular enzymes are released into the soil and fixed on clay and humic colloids through various mechanisms (Rao et al., 2014). Enzymes can be secreted by living microorganisms or released upon cell disintegration as residues of plants or animals. They are known to occur in the soil in two distinct forms free enzymes or enzymes that are stabilized on clay surfaces or soil organic matter (Burns, 1982; Rao et al., 2014).

Microbially mediated processes, which are catalysed by enzymes, underlie the cycling in soil and are essential for its functions. These processes have various roles in soil health and quality, including soil decontamination by breaking down pollutants and immobilizing heavy metals, forming soil structure, and influencing plant growth positively or negatively through plant pathogens and plant growth-promoting rhizobacteria (Alkorta et al., 2003). Enzyme reaction rates can be used to assess soil processes, productivity, microbial activity, and pollutant impact. Analysing these rates provides valuable information on the functioning and health of soil ecosystems (Srinivasrao et al., 2017). Several types of research have shown that soil enzyme activities effectively distinguish between various soil management practices, such as fertilization with animal or green manure/crop residue, municipal refuse amendment, and tillage treatments. In general, soil enzymes play a role in converting energy, maintaining environmental standards, and enhancing agricultural yields (Dick et al., 1988; García-Ruiz et al., 2009).

Soil enzymes are important for breaking down organic matter and recycling nutrients through oxidation (such as dehydrogenase, hydrolase, and glucosidase) and mineralization (such as protease, amidase, urease, phosphatase, and sulfatase) (Inamdar et al., 2022).

Although soil may contain hundreds of extracellular enzymes, only a few have been employed extensively to examine soil carbon and nutrient dynamics (Shi, 2010). Among the enzymes studied extensively that catalyse organic matter decomposition and mineralization are urease, arylsulfatase, nitrogenase, β-Glucosidase, phosphatase and β-1,4-N-acetyl-glucosaminidase (Morgado et al., 2018). The importance of these enzymes will be discussed in the subsequent sections.

Dehydrogenase (DHA)

Dehydrogenases belong to the critical oxidoreductase enzymes in the soil ecosystem. They are considered indicators of overall soil microbial activity since they are present within all living microbial cells and are tightly linked with microbial oxidoreduction processes in the soil (Gu et al., 2009; Moeskops et al., 2010). Notably, dehydrogenases do not accumulate extracellularly

in the soil. DHA is involved in biological oxidation processes by facilitating the transfer of hydrogen from organic matter to inorganic acceptors (Zhang et al., 2010). The DHA activity determines the rate of transformations in the soil (Kaczyńska et al., 2015). The total DHA activity is influenced by the collective actions of several types of dehydrogenases that are part of fundamental processes, such as the respiratory metabolism, nitrogen metabolism or the citrate cycle (Wolinska & Stepniewsk, 2012).

The most common procedure used for DHA determination involves the use of tetrazolium salts, such as triphenyltetrazolium chloride (TTC), which serve as valuable indicators of electron transport system activity. Dehydrogenases present in the soil reduce TTC, leading to the formation of an insoluble red-coloured product called triphenylformazan (TPF) (Małachowska-Jutsz & Matyja, 2019; Wolinska & Stepniewsk, 2012). The amount of TPF produced can be measured using calorimetry. However, it should be emphasized that this method only produces accurate results at a neutral pH and requires the presence of calcium carbonate to buffer the soil system (Wolinska & Stepniewsk, 2012).

Arylsulfatase (ARS)

ARS is an enzyme that facilitates the release of inorganic sulfate from organic matter by catalysing the hydrolysis of aromatic sulfate esters, producing phenols and sulfate (Alkorta et al., 2003). Sulfate is often limited in soils, and arylsulfatase is critical in enhancing its availability for better plant growth and microbial activity (Alkorta et al., 2003; Vong et al., 2003). The reaction process can be expressed by the following reaction:

$$\text{phenol sulfate} + H_2O \rightarrow \text{ sulfate} + \text{phenol} \quad (3)$$

Urease (Ure)

Urease is a critical enzyme in the hydrolysis of urea, which is widely used as a nitrogen fertilizer in agriculture and a significant component in animal urine (Alkorta et al., 2003). Moreover, the urease functions as a regulator of the nitrification process. Its activity is closely related to soil organic matter and microbial biomass levels. In soils, urease can be found as a free enzyme in solution, bound to colloidal particles (mineral or organic), and inside microbial cells. It has been observed that the higher the soil organic matter content and the microbial biomass, the higher the activity of urease (Roscoe et al., 2000). Urease also enables plants to use urea as a nitrogen source. Ure can be produced from various sources, such as plant residues and roots, animal waste or soil microbes. Bacteria and plants utilize the conversion of urea to ammonia and CO_2 as a source of nitrogen or carbon (Piotrowska-Długosz, 2019). The reaction is represented as:

$$\text{Urea } + H_2O \rightarrow \text{ } CO_2 + 2\ NH_3 \quad (4)$$

Urea hydrolysis can lead to nitrogen losses and the accumulation of nitrite and ammonia, causing several problems. Once hydrolysed, urea can be nitrified and leached from agricultural soils to surface and groundwater, making urease activity an essential index of organic nitrogen mineralization (Dharmakeerthi & Thenabadu, 2013; Piotrowska-Długosz, 2019).

Phosphatase (Phos)

An enzyme phosphatase characterizes a broad group of enzymes that hydrolyse phosphate ester, releasing phosphate that microorganisms and plants can utilize. As an extracellular enzyme, phosphatase plays a role in mineralising organic P into phosphate by breaking down phosphoric (mono) ester bonds. This process converts organic phosphorus compounds into inorganic forms ($HPO_4{}^2$, H_2PO_4). Phosphatase exhibits a low substrate specificity, allowing it to act on a diverse range of structurally related substrates. Acid and alkaline phosphatases are the two extensively studied soil phosphatases found mainly in acidic and alkaline soils, respectively (Nannipieri et al., 2011; Shi, 2010).

$$\text{phosphate monoester} + H_2O \rightarrow R - OH + \text{phosphate} \quad (5)$$

Phos come mainly from soil microorganisms but can also originate from plant cells in the rhizosphere and detritosphere. These enzymes convert organic P into phosphate through the hydrolysis of phosphoric (mono) ester bonds. Phosphatase activity is influenced by changes in the composition of the microbial community and is induced by P deficiency (Nannipieri et al., 2011).

β-Glucosidase (GLU)

GLU is an enzyme involved in the decomposition of cellulose and other β -1,4-glucans, whose primary role is the hydrolysis of cellobiose to glucose for soil microorganisms (Sinsabaugh et al., 2008). It is sensitive to soil and residue management changes and can be an early indicator of changes in soil organic carbon. Increasing BG activity indicates the ability to break down plant residues and enhance crop nutrient availability. The activity of β-Glucosidase is linked to several soil functions, such as nutrient cycling, biodiversity, filtering and buffering, and physical stability and support, within the framework of soil management assessment. (Stott et al., 2010).

$$\text{glucoside} + H_2O \rightarrow \beta - D - \text{Glucose} + R - OH \quad (6)$$

N-acetyl-β-D-glucosamidase (NAG)

NAG is with other enzymes involved in breaking down chitin, peptidoglycan and other glucosamine polymers connected by β-1,4-bonds (Sinsabaugh et al., 2008). The hydrolyses of chitin, as a critical constituent of insect and fungal cells, is crucial for carbon and nitrogen cycling because it facilitates the conversion of chitin into amino sugars, which represent a major source of mineralizable nitrogen in soils (Ekenler, 2002). Therefore, NAG activity is an asset in the cycling of both carbon and nitrogen in the soil (Parham & Deng, 2000). Activities of NAG may be involved in N-acquiring activities of microorganisms and the biological control of plant pathogens (Parham & Deng, 2000; Sinsabaugh & Moorhead, 1994). The activity of NAG is substantially related to nitrogen mineralization in long-term nitrogen-fertilized cropping systems and may serve as an indicator of nitrogen mineralization in soils (Stott et al., 2010).

Nitrogenase

An enzyme nitrogenase is responsible for converting atmospheric nitrogen to ammonia, making it available to living organisms. This enzyme is found in nitrogen-fixing bacteria, which conduct this process. Therefore, it is important to fix nitrogen for soil biological activity (Hoffman et al., 2014). Nitrogenase activity in the soil depends on the ecological conditions and specific N fixation capabilities of certain microorganisms and plant genotypes. These factors can vary under different climatic conditions. However, each plant's degree of nitrogenase activity is specific(Egamberdieva & Kucharova, 2008).

$$N_2 + 8\,e^- + 8\,H^+ \rightarrow 2\,NH_3 + H_2 + P_i \quad (7)$$

2.4.7 Materials to improve soil quality

Fertilizers and soil amendments are commonly used materials that enhance the qualities of agricultural soils and increase the productivity of crops. They effectively improve soil fertility by enhancing plant nutrient and water availability, reducing soil dryness, promoting microbial activity, and enhancing nutrient absorption. Furthermore, soil amendments can improve soil structure by strengthening the stability of soil aggregates, modifying bulk density, and improving the relations between soil and water in the soil (El-Alsayed & Ismail, 2017; Tejada et al., 2009).

Their application in the soil can enhance other ecosystems by reducing heavy metal mobility and toxicity, limiting fertilizer and toxic substance migration, lowering soil salinity, stabilizing pH, promoting CO_2 fixation, and reducing GHG emissions (Li et al., 2012; Parmar et al., 2016; Sun et al., 2019).

The four primary categories of soil amendments are as follows:

- organic (plant residues, such as livestock manures, slaughterhouse waste, compost, plant residues or biochar, fertilizers),
- inorganic (lime, gypsum, clay minerals, potassium sulfate and chloride, sand),
- synthetic (fertilizers, superabsorbent polymers, pesticides and herbicides, hydrogels),
- and microbial amendments (inoculants of mycorrhizae or other microbes) (Abbott et al., 2018; Bulluck et al., 2002; Garbowski et al., 2023).

Organic matter is widely used as natural fertilizer in agriculture due to its carbon abundance and contributes to sustaining a balance of CO_2 in the environment (Parmar et al., 2016). Increasing the concentration of organic matter enhances various soil properties, including retention capacity, aggregation, structure, mechanical strength, soil compaction, and fertility (Eden et al., 2017). The long-term use of organic soil amendments contributes to the amplification of carbon sequestration in the soil and helps to improve food safety (Parmar et al., 2016). Additionally, the amount of organic amendment and its quality are essential variables influencing soil microbial biomass (Tu et al., 2006). There are manure, slurry, and plant residues among conventional soil amendments. However, alternative organic materials, such as sewage sludge and green or municipal waste, can be composted or pyrolyzed (biochar) into soil amendments (Eden et al., 2017).

While incorporating organic supplements into the soil can offer several benefits, it may also have adverse environmental effects. Specifically, applying natural organic additives to the soil has been shown to substantially increase the emission of nitrous oxide (N_2O) and methane (CH_4) gases (Sun et al., 2019). Using organic waste directly as a soil supplement without prior stabilization can pose significant environmental risks. It can negatively impact soil microbial activity, nutrient immobilization, plant growth, and surface water quality and increase the presence of pathogenic microorganisms (Garbowski et al., 2023).

Biochar

Biochar is a carbon material produced under controlled conditions as the result of pyrolysis (i.e. thermal decomposition in a partial or anaerobic condition) of different types of biomass or organic solid waste (Hunt et al., 2010). Pyrolysis of biomass into biochar reduces waste volume, minimizes the risk of pathogens and pollutants, and increases carbon stability, reducing greenhouse gas emissions from waste (Wang et al., 2012).

Organic biochar production materials include wood, animal manure, sewage sludge, and agricultural crop residues (Ali et al., 2022). The intended application of biochar is determined by its physiochemical characteristics, which are influenced by the type of feedstock and the temperature conditions used during production (Sharma et al., 2020). Lower production temperatures yield more biochar, while higher temperatures decrease biochar yield and increase ash and carbon content (Domingues et al., 2017; Jindo et al., 2014).

Biochar improves soil fertility, enhances crop yield, mitigates climate change by carbon sequestration, reduces the leaching of nutrients, enhances water quality, and reduces heavy metal toxicity. Its ability to absorb positively and negatively charged compounds decreases the leaching of nutrients. Also, it can alter the soil's microbial community, which affects the cycle of nutrients and crop growth. Applying biochar and compost with biochar improves soil health and physical and biochemical properties (Das & Ghosh, 2020; Palansooriya et al., 2019; Sohi et al., 2010).

Other soil amendments often accompany the application of biochar to the soil. For instance, adding biochar to compost has additional benefits, including increasing oxygen levels and microbial activity, reducing N and C loss during composting, accelerating the decomposition of organic matter, and reducing greenhouse gas emissions (Wang et al., 2012).

Biochar has characteristics such as stability, high carbon content, a large specific surface area, microporosity, and strong sorption capacity (Li et al., 2019). Due to its resistance to decomposition, biochar has the potential to persist in the environment for extended periods and contribute positively to soil carbon sequestration (Ennis et al., 2012). Biochar's surface and sorption properties (Stewart et al., 2013) make it capable of reducing greenhouse gas emissions and removing various organic and mineral pollutants, such as agrochemicals, antibiotics, heavy metals, ammonium, or hydrogen sulfide (Shang et al., 2016).

Sewage sludge

Sewage sludge is produced from the wastewater treatment process, particularly during the sedimentation of solid particles. Using sewage sludge in agriculture can be advantageous or hazardous to the soil. The positive aspects of using sewage sludge as soil amendment come from its high levels of organic matter (approximately 40%), nitrogen (4%), and phosphorus (2%). Moreover, it enhances the soil's porosity, water retention capacity, and soil aggregate stability (Bueno et al., 2011; Ghacha et al., 2020).

However, sewage sludge can also be problematic, as it can contain a range of contaminants, including heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), surfactants, hormones, and other substances that may pose a threat to the environment and human health (Ghacha et al., 2020; Kominko et al., 2018; Theodoratos et al., 2000; Yang et al., 2018). In addition, using sewage sludge as a soil amendment may adversely impact microbial activity (Bueno et al., 2011).

Plant residues and their derivatives

Crop residue addition to the soil is vital for achieving higher levels of soil organic along with fertilization, as it helps in preserving soil moisture, enhancing soil structure, and minimizing erosion (Parmar et al., 2016; Tu et al., 2006). Mulching can significantly reduce soil erosion during heavy rain events (Tu et al., 2006). One effective mulching material is straw, which improves soil fertility and water retention, adds nitrogen and carbon, and increases microbial activity (Tu et al., 2006; Xia et al., 2014). Another example of soil amendments is residues from sugarcane harvesting. These residues provide a layer of protection to the soil, enhancing its physical and biological properties, improving water retention capacity, and acting as a nutrient source for plants (Prado et al., 2013).

The addition of biochar in temperate agriculture can affect microbial dynamics through physical and chemical changes in soil structure, pH, and stoichiometry. Biochar can enhance soil water retention capacity by increasing surface area and porosity, which provides suitable habitat and protection for microbes. Moreover, biochar's high surface area and reactivity can attract ions and organic compounds, creating potential sites for microbe-substrate interactions (Geisseler & Scow, 2014).

Livestock manures

For centuries, solid and liquid livestock manures (slurry) have been recognized for their positive impact on soil quality and plant growth. The application of manure, either alone or in combination with fertilizers, has been found to increase the sequestration time of carbon (Garbowski et al., 2023). Despite their benefits, using livestock manures in agriculture can cause environmental issues, such as nutrient and toxic compound leaching, nutrient loss during storage, greenhouse gas emissions, ammonia volatilization, odours, and pharmaceutical contamination. Antibiotics and hormones in the slurry can lead to antibiotic-resistant bacteria and endocrine-disrupting compounds in the environment (Garbowski et al., 2023).

Compost

Composting comprises the humification and stabilisation of organic wastes (e.g. sewage sludge, manure, municipal solid waste, and green waste) (Huang et al., 2016). Composting is one of the methods for reducing the harmful effects (accumulation of antibiotics and hormones) of using live-stock manures and other organic wastes (e.g. sewage sludge) (Huang et al., 2016; Li et al., 2012). The application of compost prepared from organic wastes can enhance soil fertility and crop production (Li et al., 2012). Furthermore, it can increase soil organic matter content, improving physical properties (water holding capacity, infiltration rate, aeration, and porosity) and stimulate the growth and diversity of soil microorganisms, increasing their biomass and activity. As compost decomposes, it can release a substantial amount of inorganic nutrients such as N, P, K, Ca, and Mg in a form that plants can easily absorb through their roots (El-Alsayed & Ismail, 2017; Irshad et al., 2013).

Moreover, compost can protect the soil against erosion. The mineral additives in the composting process are used to reduce the availability of heavy metals, increase microbial activity, and improve the composting process and the quality of the final product (Garbowski et al., 2023; Li et al., 2012). The immobilisation of heavy metals in agricultural soil and the decrease of their environmental and ecological impact are significantly influenced by mineral ions, humic compounds, and microorganisms present in compost (Huang et al., 2016).

2.5 The impact of biodegradable polymers on the soil quality and plant growth

As already discussed, extensive research demonstrates that using soil amendments can improve the growth and yield of plants while protecting against harmful agents (Zhou et al., 2021). Petroleum-based and biodegradable polymers, including micro- and nanoparticles, can quickly move through the soil matrix and contaminate groundwater (Liwarska-Bizukojc, 2021). Due to widespread transportation, plastic particles can be widely distributed and present in various ecosystems, such as marine and freshwater, as well as in soil industrial and urban locations (Nizzetto et al., 2016). In fact, they have been discovered on shorelines of remote islands (Rillig, 2012) or in high mountain areas (Scheurer & Bigalke, 2018).

Bioplastics impact both the non-living and living components of the terrestrial ecosystem. They can alter the chemical composition and structure of the soil, leading to disturbances in the water balance and cycle (Liwarska-Bizukojc, 2021). According to Wan et al. (2019), the presence of plastic films led to an increase in the rate of soil water evaporation due to the creation of channels that facilitated water movement. Additionally, bioplastics cause desiccation cracking on the soil surface (Wan et al., 2019).

To this date, there has been a limited number of studies reporting the impact of biodegradable polymers on soil pH. The effect on the soil pH is generally drawn by examining existing research on the effects of conventional plastics on soil properties. However, even these studies are inconsistent. While some studies have suggested that certain plastics, such as PE and PET, can lead to a decrease in soil pH (Bandow et al., 2017; Boots et al., 2019), others have reported opposing trends, such as for HDPE (Jia et al., 2020; Qi et al., 2020).

Bioplastics can affect the water cycle in the terrestrial environment, with certain types of polymers impacting plant evapotranspiration rates. The water-holding capacity of microplastic-treated soils can increase, leading to greater water availability, which may be further reduced by plant growth (de Souza Machado et al., 2019; Liwarska-Bizukojc, 2021). Based on the research by de Souza Machado et al. (2019), microplastics can affect plant growth, elemental composition, root traits, and soil microbial activities. Particle type influences the extent of the impact, with microplastics similar in shape to soil particles causing less pronounced effects. The results indicate that microplastic contamination in the soil can harm plant performance, agroecosystems, and terrestrial biodiversity (de Souza Machado et al., 2019). Biodegradable polymers can affect soil biota directly or indirectly. However, the studies primarily focused on plants and microbiota, with minimal data available on their impact on soil fauna (e.g. nematodes, springtails). Additionally, biopolymers can cause delayed seed germination and abnormal radicles/hypocotyls (Balestri et al., 2019; Liwarska-Bizukojc, 2021). Root and stem growth can be inhibited or stimulated, and nanoparticles can accumulate in plant organs (Bosker et al., 2019; Liwarska-Bizukojc, 2021).

Conversely, conventional nanoplastics may reduce soil microorganism biochemical activity (Liwarska-Bizukojc, 2021). Biodegradable polymers PLA and corn starch-based materials were tested for their impact on soil microorganisms, specifically soil microbial nitrification. PLA biodegradation did not affect soil microbial nitrification, as reported by Satti et al. (2018). Bettas Ardisson et al. (2014) observed in their study of corn starch-based biodegradable polymers and copolyesters. Biodegradable plastics did not inhibit soil nitrification and

increased ammonium nitrogen depletion (Ardisson et al., 2014). In another study, Arcos-Hernandez et al. (2012) investigated the biodegradation rates of PHBV and examined the effect of the degradation products on microbial activity. The soil extracts tested during PHBV degradation were non-toxic to observed bacteria (Arcos-Hernandez et al., 2012). These affirmative results indicate that biodegradable polymer materials may be used in sustainable agriculture.

Under favourable soil conditions, such as a high organic matter and nutrient content, microbial activity, rapid C turnover, and water saturation, biodegradable plastic can quickly degrade in weeks to months (Kawashima et al., 2019). However, plastic particles in degraded soils with low OM can induce undesirable soil aggregation, reducing OM accessibility to microbes, altering soil nutrient proportions, and decreasing primary plant production. This results in reduced soil quality, productivity, and SOM content. Biodegradation of bioplastics then relies on exploiting other nutrient sources in soils, including those usually used by other biota, such as plants (Yao et al., 2022; Zhou et al., 2021).

The production of biodegradable polymers like P3HB is on the rise due to climate change, as these materials have a minimal or non-existent negative impact on the environment (Volova et al., 2017). They possess favourable characteristics such as biodegradability, biocompatibility, flexibility, and thermoprocessibility, making them attractive to various industries (Alcântara et al., 2020). Biodegradable plastics like PHA are used in agriculture and as packaging materials to minimize plastic waste and soil pollution (Amelia et al., 2019; Peelman et al., 2013). However, the impact of microbioplastics on soil-plant interactions, particularly on the structure and function of soil microbial communities in agroecosystems, is poorly understood (Zhou et al., 2021).

PHA can alter the metabolic status of microbial communities, leading to increased turnover of native organic matter and impacting soil carbon and nutrient cycling (Kuzyakov, 2010; Zang et al., 2020). Soil bacteria are more responsive to changes in carbon availability than fungi, potentially leading to long-term effects on soil ecosystem services (e.g. carbon storage, nutrient cycling, and pollutant attenuation) (Barnard et al., 2013; Zang et al., 2020; Zhou et al., 2021).

Despite being rich in carbon, PHA are deficient in nutrients such as nitrogen and phosphorus, which can change the composition and function of microbial communities during biodegradation (Volova et al., 2017). In particular, the degradation of carbon-rich residues is frequently associated with the immobilisation of N and P, a process that can negatively impact plant growth by increasing competition between plants and soil microorganisms for these nutrients (Qi et al., 2020; Song et al., 2020; Zang et al., 2020).

Until now, only a limited number of studies have examined how to investigate the impact of PHB amendment on plant growth in soil (Dahal et al., 2020). According to the findings of Garrison et al. (2016), PHA, given their biological origin, are conventionally considered carbon neutral. However, this assumption is based on the premise that PHA do not induce positive priming of soil organic matter, nor do they increase N_2O and CH_4 emissions, both of which could potentially offset any carbon sequestration advantages of PHA (Zhou et al., 2021).

Research on PHA by Zhou et al. (2021), specifically poly(3-hydroxybutyrate-co-3-hydroxy valerate) (PHBV), in soil has shown that it is readily biodegradable by soil microorganisms, leading to a higher growth rate and activity of microbial biomass in the soil compared to bulk soil. Additionally, adding PHBV to the soil altered the species diversity and increased the abundance of specific bacterial taxa. PHBV created hotspots where carbon and nutrients were processed more efficiently due to the increased microbial biomass and activity (Zhou et al., 2021). While recent studies have shown divergent effects of microplastics on soil microbial communities, such as activation (Liu et al., 2017), suppression (Fei et al., 2020) or remaining unchanged (Zang et al., 2020), it is still unclear how biodegradable microplastics affect microbial functions and below-ground carbon processes; therefore, further investigation is necessary (Zhou et al., 2021).

Another study by Qi et al. (2018) was conducted to determine the effects of low-density polyethylene and starch-based biodegradable plastic mulch films on wheat growth when mixed with soil. The results showed that macro- and micro-sized plastic residues negatively impacted above and below-ground wheat development. Biodegradable plastic had a more substantial negative effect than polyethylene.

However, most beneficial to the aims of this thesis is the study by Brtnicky et al. (2022) which investigates the effect of P3HB on the soil and the effect on plant growth. The findings indicate that the soil treated with P3HB increases microbial activity, as the microorganism preferentially uses P3HB as a carbon source. This can lead to reduced nitrogen in the soil and an inhibition of plant growth. Furthermore, biodegradation of P3HB in the soil with low nutrient content can temporarily replace soil organic matter as a carbon source for the microbial community. It can be attributed to changes in the microbial community composition towards those that promote P3HB degradation. Additionally, it suggests that P3HB has the potential to influence soil and plant health in complex ways that need to be further examined (Brtnicky et al., 2022).

One study showed no negative effects of PHBV on maize (Dahal et al., 2020), while another study found an increase of the 3-hydroxybutyrate (3-HB), a degradation product of P3HB, resulted in an increased expression of genes involved in chromatin remodelling and activation of DNA demethylation. Excess 3-HB could affect the plant's ability to cope with abiotic stress. In a study of transgenic plants designed to produce PHB, high levels of PHB production have reduced seedling survival (Mierziak et al., 2020), suggesting adverse effects on plant development (Malik et al., 2015).

In summary, microbioplastics within the soil can potentially disrupt ecological functionality and biogeochemical cycling, including critical processes such as soil organic matter decomposition (Zhou et al., 2021). However, limited research focuses on the synergistic effects of soil amendments with P3HB in developing more sustainable fertilisers for agriculture. Despite being promoted as a potential solution to reduce microplastic residues in terrestrial ecosystems and is considered a substitute for various applications, the environmental impacts of PHA have not been thoroughly investigated (Zhou et al., 2021).

Aim of the work

The thesis aims to investigate the impact of poly-3-hydroxybutyrate (P3HB) biodegradation on soil properties and plant growth by conducting a series of experiments and, thus, evaluating its potential use in soil applications.

The consequences of the presence of biodegradable plastics in the soil have not been thoroughly studied, despite their growing use as an alternative to conventional plastics in the agro-industrial sector. Their effects are still rather assumed from studies on conventional plastics. This work follows the research conducted by Brtnicky et al. (2022), who studied the effect of P3HB on soil properties and plant growth and observed a boost of soil microorganisms growing connected with a negative effect of biodegradation on plant growth. This study investigates not only the effects of P3HB but also considers the impact of other organic soil additives such as compost, biochar, manure and separate that may potentially mitigate the adverse effects of P3HB observed in previous studies.

The working hypothesis of the work is:

1. The biodegradation of P3HB negatively influences plant growth, but the growth can be improved by the addition of amendments.
2. The rate of P3HB biodegradation is supported by the addition of organic amendments.

To achieve the objectives of the thesis and verify the hypotheses, various approaches will be employed:

i. the chemical and physical characteristics of the soil, such as pH and dry matter content;
ii. the impact on plant growth will be evaluated, specifically on the aboveground biomass, to assess any potential inhibition of plant growth;
iii. the enzymatic assay will be used to study the microbial community;
iv. the microrespirometry will be used as an effective method of CO_2 release monitoring to investigate P3HB's biodegradation in soils, including its reaction rate and the amount of carbon transformed by microorganisms;
v. thermogravimetry will be utilized to investigate whether the degradation was complete and to assess any additional impact of P3HB on soil properties.

3 EXPERIMENTAL PART

The experimental soil was collected from the topsoil (at a 0–15 cm depth). The sampling location was near the town of Kroměříž in the Czech Republic, and the soil was identified as arable soil. Based on the Czech Taxonomic Soil Classification System, the selected soil was classified as a cambisol (Sáňka et al., 2018). The soil analysis was conducted at the Faculty of AgriSciences at Mendel University in Brno and the Faculty of Chemistry of the Brno University of Technology.

The soil had a low humus content of 1.43 % (typically between 1–6% for cambisols) and was of low quality at 0.56. This is normal for cambisols at an altitude of around 100m below sea level, where humus content is expected to be less than 3 %. The soil is moderately acidic with a pH_{KCl} of 4.86. It has a light texture and low clay content, with 22 % of particles smaller than 0.002mm and 30 % smaller than 0.01mm. This indicates sandy soil. The soil has a low cation exchange capacity (90 $mmol_+/kg$) and high bulk density (1.70 g/cm^3), which is typical for mineral soils. It also has low soil porosity at 11.65 %. The soil's airiness is high (11. 65%) and has low porosity. The total nitrogen was estimated to be 0.21 %.

The soils originated from the experiments in which the soil was amended with six different soil amendments such as compost, biochar 15 t/ha, biochar 45 t/ha, manure and solid digestate fraction (separate) in 2017. After sampling, the soil was sieved through a 2 mm mesh sieve. The soils were put into the pots, and poly-3-hydroxybutyrate powder was added. The final soil samples contained the sole amendment and the mixture of amendments with 1%, a combination of P3HB. The pure biodegradable polymer (i.e. without additional additives) P3HB was provided in a powdered form with a particle size smaller than 80 μm by TianAn Biologic Materials Co., Ltd. (Ningbo City, China) with the trade name ENMAT Y3000. P3HB microparticles had spherical or spherical-like shapes. The reported contact angle is around 70° and approximately 81°, indicating that the surface of P3HB is slightly hydrophobic (Pompe et al., 2007). The soil properties in terms of fertilizing inputs of individual variants are contained in Tab. 1. It includes the amount of organic amendments (t/ha) added to the soil and the macronutrient content (g/kg).

Tab. 1: Overview of experimental soil treatments and their corresponding fertilizing inputs prior to the addition of P3HB.

Treatment / Variants	Fertilizing 2017 [t/ha]	N^*_{input} [g/kg]	P^*_{input} [g/kg]	K^*_{input} [g/kg]	Fertilizing 2019 [g/kg]	N_{input} [g/kg]	P_{input} [g/kg]	K_{input} [g/kg]
Control soil	–	–	–	–	–	–	–	–
Compost	50	190	45	75	50	225	45	75
Biochar 15 t/ha	15	240	135	450	–	–	–	–
Biochar 45 t/ha	45	80	45	150	–	–	–	–
Manure	50	90	35	225	30	70	50	130
Solid digestate	40	200	70	190	30	160	50	135

*N_{input}, P_{input}, K_{input}: the amount of nitrogen (N), phosphorus (P) and potassium (K) added to soil with amendments.

3.1 Pot experiment

The study involved a pot experiment to evaluate the effect of P3HB on soil properties and plant biomass, specifically with the maize seedlings (*Zea mays L.*). The pot experiment was conducted for eight weeks in a growth chamber in controlled conditions, including full-spectrum LED lighting (intensity 370 $\mu mol \cdot m^{-2} \cdot s^{-1}$); photoperiod 12 h; temperature 16/23 °C (night/day) and the relative humidity 70/49% (night/day). Then one kg of every experimental soil variant was filled into experimental plastic pots (volume 1 L) with three maize seeds sown in each pot. A total of 12 experimental variants were established, consisting of six variants shown in Tab. 1, each of which was co-amended with P3HB and replicated in three pots. The soil was watered with 50 ml of demineralized water twice a week. After ten days, the most robust plant per pot was retained. The pots were rotated once weekly after being randomly placed in the growth chamber.

3.2 Soil analysis

Upon completion of the experiment, the aboveground maize was cut at ground level. Soil samples were obtained from each pot to determine their corresponding properties. The fresh aboveground biomass (AGB) of maize was then dried at 60 °C to determine dry AGB. For the determination of pH and thermal analysis, air-dried soil samples were used. The freeze-dried soil was further used to analyse enzyme activity; samples stored at 4 °C were subjected to basal and substrate-induced respiration.

3.2.1 Determination of pH, soil dry matter content and aboveground biomass

The fresh aboveground biomass of maize was dried (at 60 °C) in an oven at a constant temperature, and the dry AGB was determined by gravimetry on analytical scales. The dry matter content in the soil was determined using the ISO 11465:1993 standard procedure, which involved drying fresh soil samples in the oven (105 °C) until a constant weight was achieved by removing excess moisture (International Organization for Standardization, 1993).

The measurement of soil pH followed ISO 10390:2005 standards and involved the use of a glass electrode in a 1:5 (volume fraction) mixture of soil and water, resulting in a pH measurement in water and in 1 mol/L potassium chloride solution (International Organization for Standardization, 2005).

3.2.2 Soil enzyme activity assays in soil samples using colourimetric substrates

Enzyme activity in freeze-dried soil samples was assessed according to the ISO 20130:2018 standard (International Organization for Standardization, 2018). Multiple hydrolase enzymes, including dehydrogenase (DHA), arylsulfatase (ARS), β-glucosidase (GLU), N-acetyl-β-D-glucosamidase (NAG), phosphatase (Phos), and urease (Ure), were measured simultaneously using colourimetric compounds.

3.2.2.1 Materials and methods

- Deionized (distilled) water (dH_2O)
- Tris(hydroxymethyl)aminomethane hydrochloride (100 mmol/L)
- Calcium chloride dihydrate (0.5 mol/L), $CaCl_2 \cdot 2H_2O$
- Salicylate reagent
- Cyanurate reagent

- A stock solution of NaOH (5 mol/L)
- Enzymes (DHA, ARS, GLU, NAG, Phos, Ure)
- Sieves (mesh size 2 mm)
- Balance
- Multi-well microplates (96 wells)
- Automatic dispenser
- Orbital shaker
- Multichannel micropipettes (50 µL, 200 µL)
- Magnetic stirrer
- Incubators
- Place centrifuge
- Infinite M Nano microplate reading spectrophotometer with a single-mode microplate reader and monochromator optics

3.2.2.2 Procedure

A plastic bottle was filled with 2.5 g of lyophilised sample and topped with 12.5 mL dH_2O. The resulting soil solution was placed in the orbital shaker and subjected to 10 min agitation (180 oscillations/min). The soil solution was distributed on 96 micro-well microplates in two sets, with 125 µL for all samples except urease (which received 50 µL). The bottle containing the soil solution remained in contact with the stirrer to ensure homogeneity during pipetting. The enzyme was dosed into the same well as the soil sample suspension. The substrate solution was primed into the same well after the soil solution, with 25 µL for all samples except urease (which received 40 µL), using a 12-channel multi-pipette substrate solution. The enzyme substrate was dispersed in three rows, with the fourth row omitted as a control. For urease, the substrate was diluted with dH_2O (150 µL) before dosing, and the enzymatic substrate was dosed in three lines, with the fourth line supplemented with dH_2O (40 µL). After the enzyme substrate, the microplate was placed in an incubator. After incubation, $CaCl_2 \cdot 2H_2O$ (25 µL) was added to each well according to the protocol to stop the reaction (except for Ure). Tris(hydroxymethyl)aminomethane hydrochloride (100 µL) was added. The enzyme substrate (25 µL) was injected into the control lines (fourth column in order). The next step was centrifugation (20 °C, 1500 rpm, 10 min). After centrifugation, a sample solution (200 µL) was pipetted into the new multi-well microplate, with the remaining soil settled on the bottom of the well. The microplate was then placed on a spectrophotometer (405 nm). The enzymatic reaction was terminated for urease samples by adding salicylate (40 µL) to each well and allowing it to incubate for 3 minutes. Subsequently, cyanurate solution (40 µL) was added to each well, and then the multiwell microplates were placed in the incubator (30 min, 25 °C). Subsequently, the microplates were subjected to centrifugation. After centrifugation, 200 µL of the sample solution (with the soil settled at the bottom of the well) was extracted and measured using a spectrophotometer (650 nm).

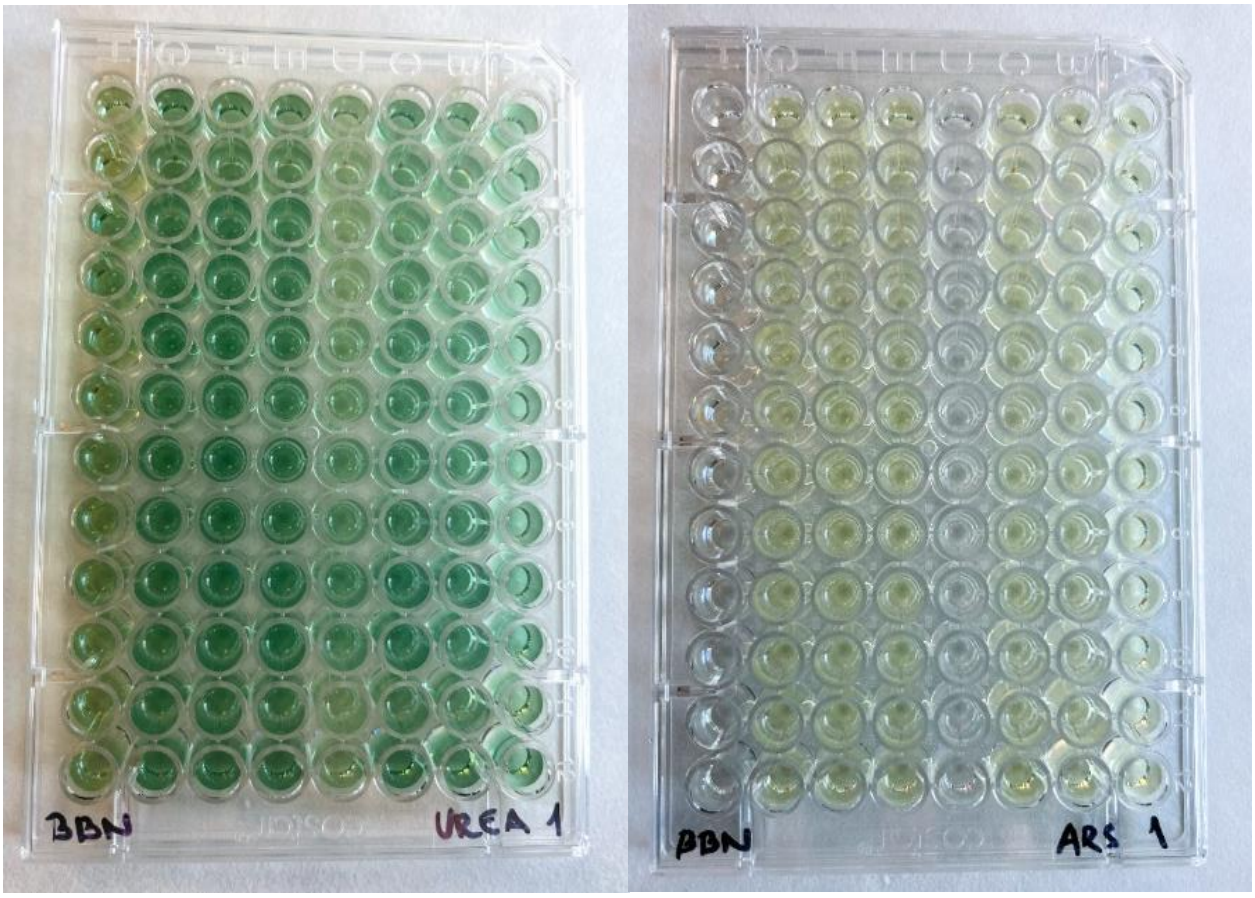

Fig. 6: Demonstration of colour changes depending on the added substrate after decomposition, Ure (left) and ARS (right).

3.2.3 Spectrophotometric assay of soil respiration

The microrespiration technique was used to measure soil basal (BR) and substrate-induced respiration (SIR) in the soil samples. This technique detects and quantifies CO_2 released or used by the microbial community and colourimetrically determines the soil respiration rates. The change can be observed as a reduction in absorbance. Measuring the production rates of individual carbon sources can provide useful information about carbon and nitrogen mineralization. The MicroResp technique can also assess the metabolic diversity of soil microorganisms by using different chemicals as carbon sources (Campbell et al., 2003; Creamer et al., 2014).

3.2.3.1 Materials and methods

- Deionized (distilled) water (dH_2O)
- Substrates:
    - D-glucose (Glc)
    - Protocatechuic acid (Pro)
    - D-Mannose (Man)
    - D-trehalose (Tre)
    - N-acetyl-β-D-glucosamidase (NAG)
    - L-alanine (Ala)
    - L-Arginine (Arg)

- Incubator
- Reaction deepwell microplates
- Detection deepwell microplates with detection agar
- Perspex sheet
- Parafilm
- Spatula
- Brush
- Sealing rubber septum
- Metal microplate clamps
- Single channel automatic pipettes (2–20 µL, 20–200 µL)
- 12-channel automatic pipette (10–200 µL)
- Plastic trays for multichannel pipette
- TECAN microplate reader
- Weighing tray

3.2.3.2 Procedure

Preparation of detection microplates

Soil samples (stored at 4–5 °C) were gradually filled into the deepwell microplates using parafilm to cover all columns other than those to be filled. The soil was evenly sprinkled on the surface of the uncovered area and gently brushed into the columns until all were filled to the rim. To expose the bottom holes of the columns, a sliding motion was used to pull the Perspex sheet. Once the column was filled, the filling device was removed from the microplate, and the microplate was weighed. After weighing, the weight and the microplate were reset on the tray, and the filling device was placed back on the microplate. In this way, all 12 columns were filled in sequentially, and each column was weighed. Once all wells in the microplate were filled, the filling device was removed, and the microplate was covered with Parafilm. Subsequently, the microplate with soil samples was incubated for 72 h (25 °C, dark environment).

Fig. 7: Preparation of detection microplates.

Preparation of reaction microplates

Microplate wells were filled with soil samples, and carbon sources were added after incubation. For the preparation of substrates (carbon sources), a concentration of 30 mg/g of soil water was used. Stock solutions with the concentrations 0.05–0.2 g/mL in dH_2O were prepared for the following substrates: Ala, Arg, Glc, Man, NAG, Pro and Tre. To calculate the amount of carbon source to be added, it was necessary to know the weight (g) of the soil in each well and the moisture content (% dry matter) of the tested soil. The distilled water was then added to the calculated volume of the substrate solution to a total volume of 25 µl. The entire volume of dH_2O (25 µl) was added in the first row for a basal respiration measurement. To the remaining wells, the diluted substrates were dispensed into the appropriate microplate wells using a 12-channel pipette. Finally, the septum detection microplate was immediately inserted and pressed into all wells of the deep-well microplate. The metal MicroResp holders were then inserted, and the two plates were pulled together and incubated in the dark for 6 hours at 25 °C (laboratory temperature). The Deepwell microplates were then separated from the detection microplate, and the agar plate was measured on a TECAN reader using the MicroResp method (At_6). Each soil sample was measured in 4 repetitions for every tested carbon source and basal respiration measurement (i.e. for every soil sample, four columns are tested with seven different carbon sources (see scheme in *Fig. 8*). The absorption (A_0) of a detection microplate was measured on the TECAN reader using the MicroResp method (570 nm). The detection microplate with the highest homogeneity was selected from an exicator. The microplates should be uniformly pink

(i.e. agar is completely regenerated) and without the presence of bubbles in wells. The % coefficient of variance (% CO_V) will be examined from the results obtained at a time "At_0". If $\%CO_2$ exceeds 5%, the microplate will be discarded, and the next one will be used.

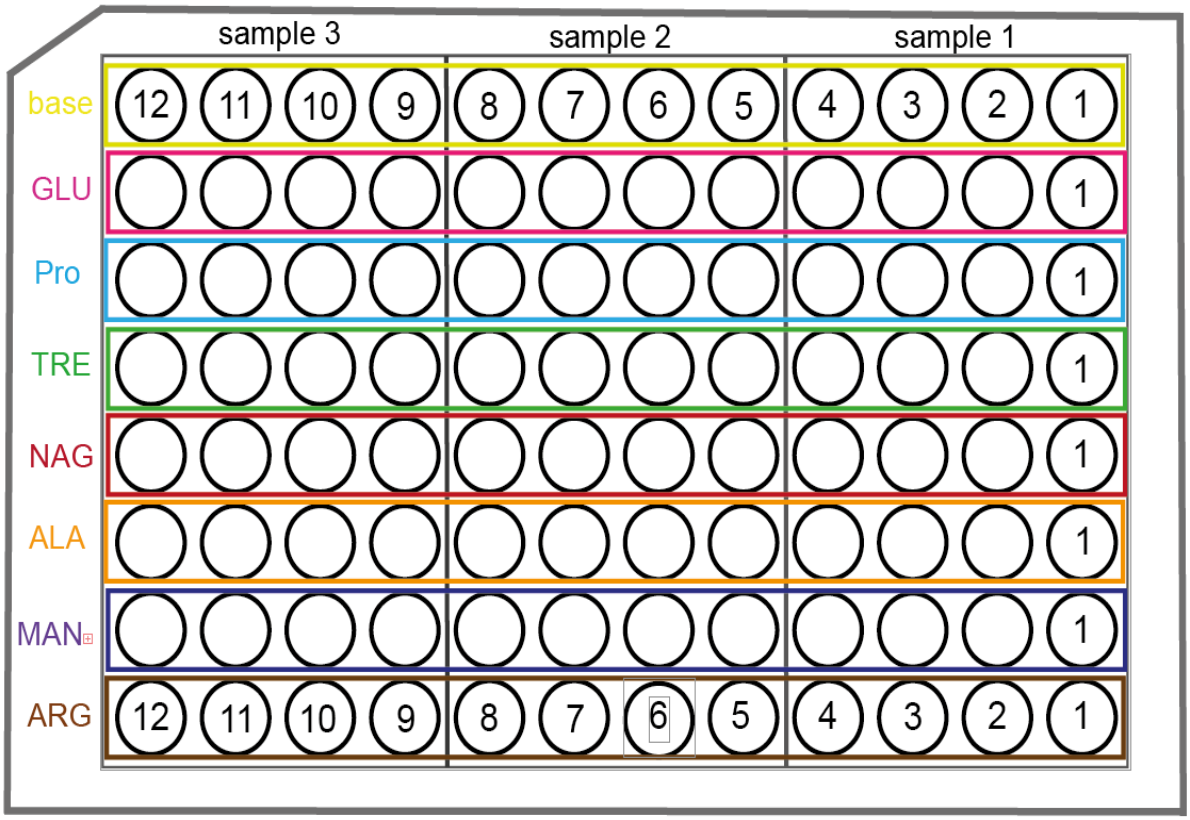

Fig. 8: Scheme of substrate filling. (Basal) with distilled water; (GLU) D-(+) Glucose; (Pro) protocatechuic acid; (TRE) D-(+)- Trehalose;(NAG) N Acetyl glucosamine; (ALA) L-Alanine; (MAN)D-Mannose; (ARG) L-Arginine.

3.2.4 Thermal analysis of P3HB in the soil and its effect on soil properties

Thermogravimetric analysis (TGA) is a method of thermal analysis that determines the mass loss (%) of a material when subjected to heating over a defined temperature range. The degradation process is terminated when a constant temperature is reached or at the programmed end temperature. In order to improve the information value of the record, the TGA analysis sometimes also involves the temperature derivative thermogravimetric (TG) curve. The resulting TG curve provides information on thermal stability, initiation temperature of degradation, weight loss rate, final residue, and chemical or physical properties of the analysed material (Plante et al., 2009).

3.2.4.1 Materials and methods

- Thermogravimetric analyser TGA 550 from TA Instruments
- TGA alumina pans
- Petri dishes

Fig. 9: Thermogravimetric analyser TGA 550 (TA Instruments/Waters Corporation, 2022).

3.2.4.2 Procedure

The analysis of soil samples was carried out using a TGA 550 thermogravimeter from TA Instruments (Fig. 9). About 200 mg of soil samples were dosed onto aluminium pans and placed on an automatic sampler. Using *TRIOS software*, parameters were set, and a program was selected to heat the samples and record any weight (mass) changes on scales within the device. The samples were heated according to the chosen program, causing weight changes to be recorded by scales within the thermogravimeter. The platinum hooks served as both a pan holder and a weight detector. Externally supplied air was used to purify the scales, and a specially modified lid attached to the autosampler was used to ensure a relative humidity of 43±2% during the analysis. The lid included a bypass connected to two Dreschel bottles to provide air with relative humidity in three directions. The first bottle was filled with it by bubbling air through a saturated potassium carbonate solution, while the second was filled with cellulose to prevent clogging and contamination. Similarly, humidified air was blown into the oven using the same bypass during analysis to maintain consistent conditions and to avoid clogging.

Data were sent directly to a computer for evaluation. Measurements of each sample were repeated at least three times. A list of parameters and their respective values for thermal analysis is presented in Tab. 2.

Tab. 2: List of parameters for TGA analysis.

Parameters	**Value**
Protective gas	Air
Protective gas rate	60 [ml/min]
Reaction atmosphere gas	Air
Reaction gas velocity	60 [ml/min]
Temperature gradient	5 [°C/min]
Initial temperature	laboratory temp. (app. 20 °C)
Final temperature	700 [°C]
Type of pan	Al_2O_3
Sample weight approx.	200 mg
Cooling time after completion	50 min
Balance flow	10 [ml/min]
Relative humidity	43±2 %

The accuracy of the experimental results may have been compromised by the inconsistent loading of samples into instrumental pans. The relatively small amount of sample used for the analysis made it challenging to achieve complete homogeneity, particularly in soil amended with P3HB. Consequently, multiple measurements of the same sample may have exhibited different concentrations of the biopolymer in the soil.

4 RESULTS AND DISCUSSION

4.1 Effect of soil amendments on physical and chemical properties

4.1.1 Assessment of soil pH

In the context of investigating the impact of P3HB addition on soil quality, it is vital to consider the possible potential alteration in soil pH resulting from P3HB addition. The results of our experiments indicate that the quantity and composition of P3HB used did not lead to a significant alteration in soil pH (as reported in Tab. 3). A decrease of approximately 0.7% in pH was observed. However, to further evaluate the effect of P3HB on the pH, an Analysis of Variance (ANOVA) test was conducted. The results indicate that the addition of P3HB had a significant effect on the soil pH, as evidenced by the significant F-value (462.44) and extremely low p-value ($< 2.2 \times 10^{-16}$) for the variant factor. This suggests that P3HB addition had a significant impact on the soil pH, and therefore, when investigating the impact of P3HB addition on soil quality, it is important to consider the potential alteration in soil pH caused by the addition of P3HB.

These findings are consistent with previous research conducted by Boots et al. (2019), which examined the impact of biodegradable polymer PLA on soil pH levels. This study found that PLA did not affect pH levels. Conversely, biodegradable mulch films such as PLA and PHA have been found to cause a minor reduction in soil pH (Sintim et al., 2019). However, only a limited number of studies have been conducted on this topic, making it difficult to compare obtained findings with others.

However, the biopolymers have the potential to release acidic or basic byproducts during the process of biodegradation, which could potentially affect the soil pH. In the case of P3HB, the biopolymer releases 3-hydroxypropionic acid (Chun et al., 2014), although its effect strongly depends on the concentration of P3HB and biodegradation conditions. Besides that, what can be assumed is that the influence of biopolymers on soil pH could be dependent on the soil type and the vegetation (Zhao et al., 2021). Moreover, it is worth considering that soil porosity, aeration, and aggregate size may also contribute to potential changes in soil pH. However, to confirm this hypothesis, further testing would be necessary, including varying amounts of biopolymer added to the soil.

Tab. 3: Determination of soil pH in two soil conditions, one without the addition of P3HB and the other with P3HB addition.

Type of amendment	Non-amended pH*	P3HB pH*
Control soil	5.50 ± 0.04	5.46 ± 0.04
Compost 50 t/ha	5.99 ± 0.01	5.91 ± 0.02
Biochar 15 t/ha	5.42 ± 0.01	5.38 ± 0.02
Biochar 45 t/ha	5.82 ± 0.02	5.76 ± 0.03
Manure	5.62 ± 0.02	5.56 ± 0.03
Separate	5.52 ± 0.02	5.47 ± 0.03

* Values calculated as average from independent replicates (n = 3) ± standard deviation.

4.1.2 Assessment of plant aboveground biomass

Another factor for evaluating the quality of P3HB-amended soil is dry aboveground plant biomass (AGB), which determines plant growth and crop yield. As shown in Fig. 10, applying P3HB and other amendments negatively impacted AGB, leading to a considerable reduction in plant biomass by approximately 85 %. Incorporating other amendments into the soil resulted only in a marginal decrease in overall biomass reduction (by 24 % average) compared to P3HB. However, the combination of P3HB with other soil amendments increased the overall biomass, although it was still lower than the control soil. The effect of compost (0.93% decrease), biochar at 15 t/ha (0.05 % decrease), and biochar at 15 t/ha (0.06% decrease) contributed to a further decrease of the overall biomass content after post-application of P3HB. A slight increase in dry biomass was observed in the presence of manure and separate, both accounting for 0.02%. Overall, the application of P3HB had an adverse effect on the content of dry above-ground biomass, leading to the inhibition of plant growth. This impact could not be mitigated even with the addition of other organic soil amendments.

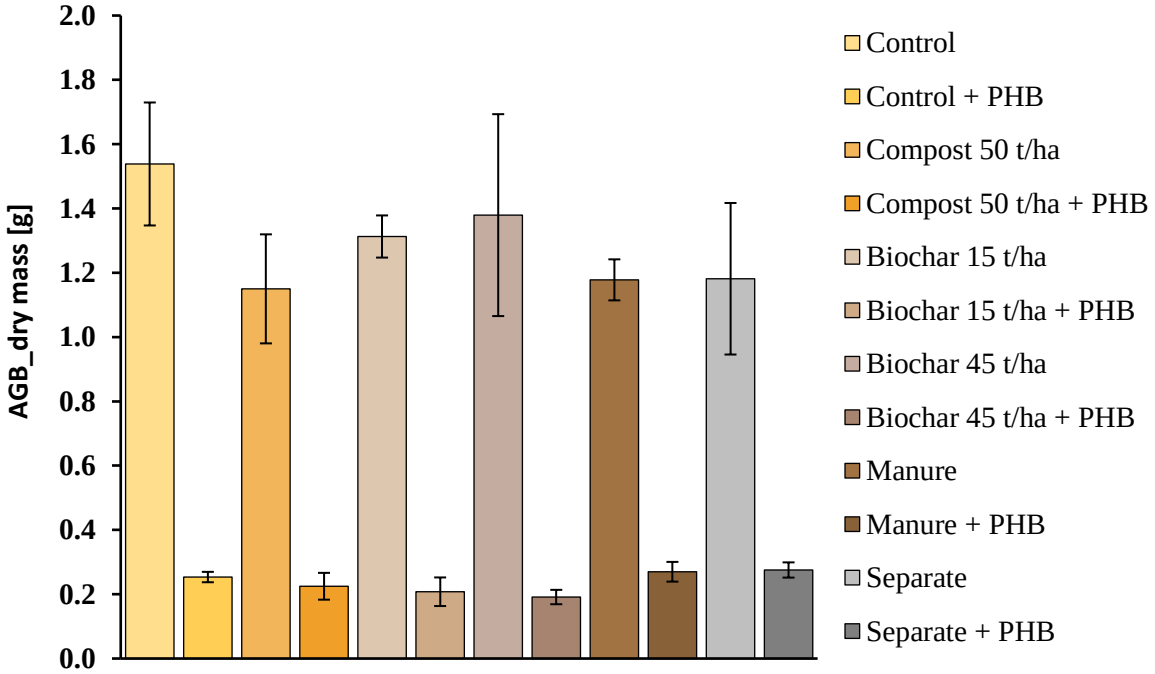

Fig. 10: Dry aboveground plant biomass (AGB). Average values of independent replicates (n = 3), error bars = standard deviation.

The findings obtained in this study regarding the reduction of above-ground biomass due to the presence of the polymer align with previous research conducted by Qi et al. (2018), Liwarska-Bizukojc (2022) or Zang et al. (2020), who also reported the negative effects of studied polymers on both above-ground and below-ground plant components. To provide an explanation for these unfavourable results, several assumptions can be considered.

Furthermore, these results are consistent with previous studies that have demonstrated the phytotoxic effects of biopolymers, with the degradation of biodegradable polymers being cited as a potential cause for this outcome (Qi et al., 2020; Qi et al., 2018; Zang et al., 2020). One possible explanation for this outcome could be the direct toxicity of P3HB particles or their

degradation products on the plants (Mierziak et al., 2020). It has been reported that 3-hydroxybutyric acid, a biodegradation product of P3HB that is produced in more significant amounts, could potentially induce soil acidification and, as a result, influence the AGB (Mierziak et al., 2020). However, it was not proven in the course of the research, as it is doubtful that rapid microbial consumption of biopolymer could lead to the accumulation of monomers (Jan et al., 2009). The obtained results could not prove this hypothesis, as the control soil was already acidic and as already discussed in section 4.1.1, the incorporation of P3HB did not result in significant pH changes.

Furthermore, the presence of additives or contaminants in the P3HB biopolymer could also potentially lead to phytotoxicity (Zhou et al., 2021). However, it is essential to note that the P3HB used in the experiment was provided in a pure form, without any additional additives. Therefore, this hypothesis can be rejected as an explanation for the observed results.

The addition of P3HB to soil may have altered other soil properties, which could explain the observed changes in plant growth and productivity. While it was acknowledged that P3HB could increase the water-holding capacity of the soil (de Souza Machado et al., 2018), its hydrophobic nature and potential to cause higher drain-off could affect water distribution and availability to plants (Brtnicky et al., 2022; Pompe et al., 2007). This can impact the dynamics of water and nutrient availability, ultimately influencing microbial activity and chemical speciation processes in the soil. As a result, the growth and productivity of plants can be negatively impacted (de Souza Machado et al., 2019).

According to the study by Silveira Alves et al. (2019), P3HB metabolism may contribute to bacterial plant growth promotion, and deleting genes involved in P3HB synthesis and degradation could reduce the bacterial ability to enhance plant growth. This suggests that the addition of P3HB to the soil could lead to an imbalance in the microbial community, with a shift towards P3HB-metabolizing bacteria, inhibiting those that promote plant growth and leading to reduced plant productivity. However, further research is needed to confirm this hypothetical explanation.

Another explanation could be the indirect impacts on plant growth resulting from changes in other soil properties or inhibition of microbial communities and nutrient availability, as proposed by Brtnicky et al. (2022). It is possible that the application of P3HB could initiate microbial immobilization of essential nutrients. This could lead to increased plant stress due to nutrient unavailability, ultimately affecting plant growth and productivity (Brtnicky et al., 2022; Zhou et al., 2021).

Apart from that, an input of organic amendment as P3HB can potentially serve as a source of carbon for microorganisms, leading to long-term effects on microbial composition, activities, and functions in the soil. When P3HB gradually decomposes in the soil, its products can enhance the activity of microorganisms to mineralize nutrients which can be either used by microorganisms as a source of energy or could result from an adverse consequence of plant-microbiota interaction, such as competition for nutrients can be taken up by plants (Pathan et al., 2020; Zhao et al., 2021). Specifically, the changes in the content of DOC and microbial biomass C due to biodegradable polymer intrusion were reported (Zhou et al., 2021). Moreover,

N immobilization was evidenced by reduced dissolved organic nitrogen and increased microbial biomass N (Zhou et al., 2021), indicating the direct influence of the addition of biodegradable polymer on carbon and nitrogen cycles. These alterations could also impact the plant growth. Consequently, the effects of P3HB on microbial activity and nutrient cycling will be further investigated through soil enzyme activity and soil respiration assays in the upcoming sections, with the aim of either confirming or rejecting the hypothesis.

4.1.3 Assessment of the dry matter content in the soil

The additional soil quality indicator that is considered valuable in the determination of the P3HB impact on the soil properties is the dry matter (DM) content of the soil. The variation in soil dry matter content may serve as an indicator of soil humidity levels (International Organization for Standardization, 1993). In this study, it was observed (see Fig. 11) that the application of P3HB alone resulted in a loss of dry matter (by 1.42 %), whereas the application of compost, biochar, manure and separate led to an increase in the content of DM (averagely by 1.13 %).

The study observed a significant increase in the DM content when P3HB was combined with other amendments, compared to its addition alone. A particularly high increase was observed in the case of P3HB combined with manure (by 2.9 %). The effect of biochar at 15 t/ha (1.68% increase), biochar at 45 t/ha (1.45% increase), and separate (0.49% increase) also contributed to the enhancement of DM content after the addition of P3HB. Furthermore, compost increased the DM to a lesser extent by 0.40 %. It should be noted that the increased DM values mentioned are relative to the effect of the biopolymer itself. However, when comparing the overall difference between the control soil sample and the soil samples containing organic amendments with post-application of P3HB, the results are not entirely positive.

The study found that the overall increase in soil dry matter content with combined amendments occurred only in specific variants, such as manure (1.55% increase), biochar at 15 t/ha (0.33% increase), and biochar at 45 t/ha (0.1% increase). In contrast, post-addition of P3HB to compost and separate resulted in a reduction of DM content by 0.95% and 0.86%, respectively.

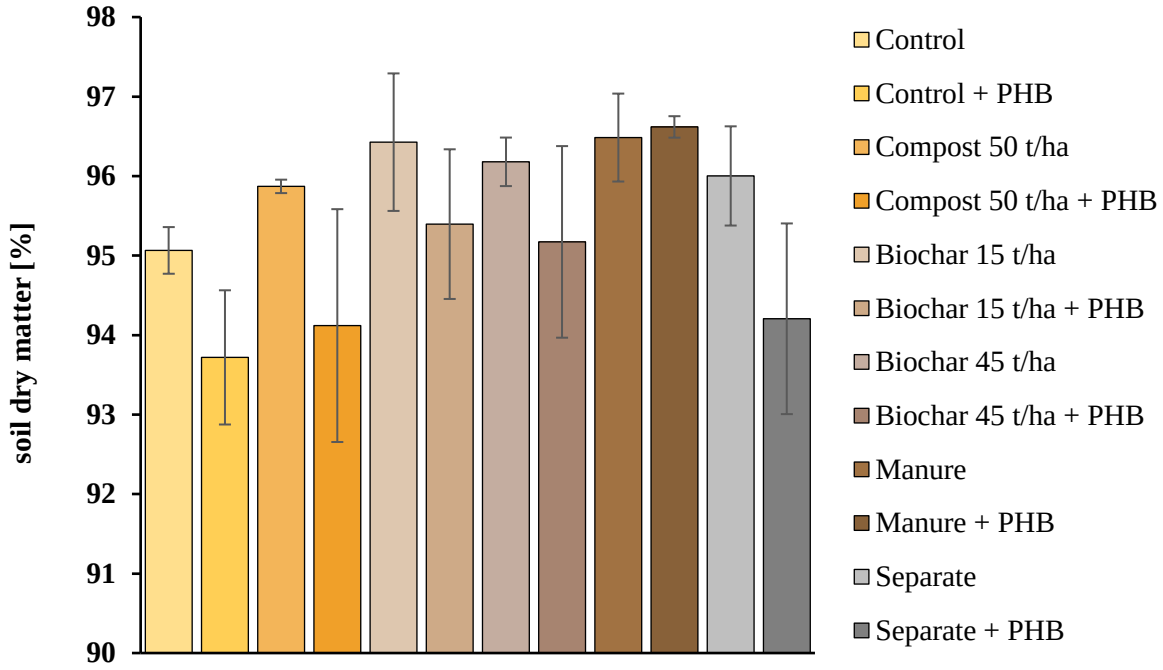

Fig. 11: Dry matter content of all tested variants. Average values of independent replicates (n = 3), error bars = standard deviation.

Based on the findings, it can be inferred that the positive effects of P3HB on soil quality, specifically in terms of dry matter content, are dependent upon the specific combination of amendments used. Moreover, it was observed that only a combination of P3HB with manure and biochar 15 t/ha resulted in more relevant mitigation of the potential negative effects of P3HB connected to DM reduction.

However, to statistically evaluate the effect of P3HB on the DM, an ANOVA test was performed. The results revealed a significant influence of the different organic amendments or P3HB on DM content in the soil, as evidenced by the F-value of 4.33 and a low p-value of 1.3×10^{-3}. Moreover, the p-value > 0.05 suggests there are significant differences between the treatment variants in terms of their effect on DM.

Due to the research gap in the area, assumptions made about the effect of biodegradable polymers on dry matter content lack empirical evidence and require further scientific investigation for confirmation. The observed results may be attributed to the initiation of thermal degradation of the polymer during the analysis drying process at 105°C, which could have resulted in alterations in the DM content. Furthermore, the different physical properties of the selected amendments could also account for the differences in their effects. For example, manure, being a natural fertilizer, may have contributed a higher nutrient content and improved water capacity, ultimately enhancing soil structure and resulting in an increase in DM (El-Alsayed & Ismail, 2017). This effect was expected to be seen with compost as well, but the opposite trend was observed. Additionally, the increase in DM may have resulted in a higher bulk density, which can be caused by soil compaction or aggregate disruption, both of which are considered detrimental to soil quality (Boots et al., 2019; de Souza Machado et al., 2019).

4.2 Effect of soil amendments on microbial properties

4.2.1 Soil enzyme activity assays

This section will discuss the impact of soil amended with P3HB along with different organic fertilizers, including manure, separate manure, compost, and biochar, on the activity of soil enzymes. This thesis focuses on soil enzymes that are essential for plant growth and participate in vital soil processes, including dehydrogenase, arylsulfatase, β-glucosidase, urease, N-acetyl-β-D-glucosaminidase, and phosphatase. However, it should be noted that measurements conducted under laboratory conditions using artificial substrates cannot replace the actual rate of enzyme processes in the soil *in situ* but give a good concept of processes occurring in soil. The results will be interpreted based on the graphical illustration of the measured data depicted in **Error! Reference source not found.**.

As with previous assays, the studies on the effects of P3HB on soil microbial properties are limited, and the discussion will be based on the knowledge derived from the analysis of conventional polymers. It has been revealed through recent studies on conventional microplastics that divergent influences on soil microbial communities and enzyme activities, the exact consequences of biodegradable polymers on soil microorganisms have yet to be fully comprehended by the scientific community (de Souza Machado et al., 2019; Fei et al., 2020; B. Liu & Thayumanavan, 2017; Zang et al., 2020).

The experimental results indicate that using P3HB as a soil amendment leads to a significant improvement in selected enzyme activities, except for GLU. The influence of the studied organic amendments, namely compost, biochar, manure, and separate, on enzyme activity is relatively modest compared to their combined effect with P3HB. Compost has demonstrated the most substantial impact among the soil amendments used. In contrast, the graphical data from **Error! Reference source not found.**of biochar demonstrate that the application of biochar alone (i.e. without the co-addition of P3HB) did not substantially enhance the enzyme activity. In each sample containing the sole biochar (both biochar15 and 45 t/ha), the enzyme activity either marginally decreased (ARS or Ure) or remained comparable to the non-fertilised soil (DHA, NAG). The addition of organic matter to soil can serve as a carbon and energy source for microorganisms, thereby increasing their activity and growth (Brtnicky et al., 2022; Garbowski et al., 2023). When organic matter is decomposed, it can result in higher nutrient content, promoting the activity of specific microorganisms that metabolize those nutrients. This can ultimately lead to improvements in soil quality and development through a higher content of available nutrients. Additionally, these nutrients can be taken up by plants, leading to increased plant growth and higher yields.

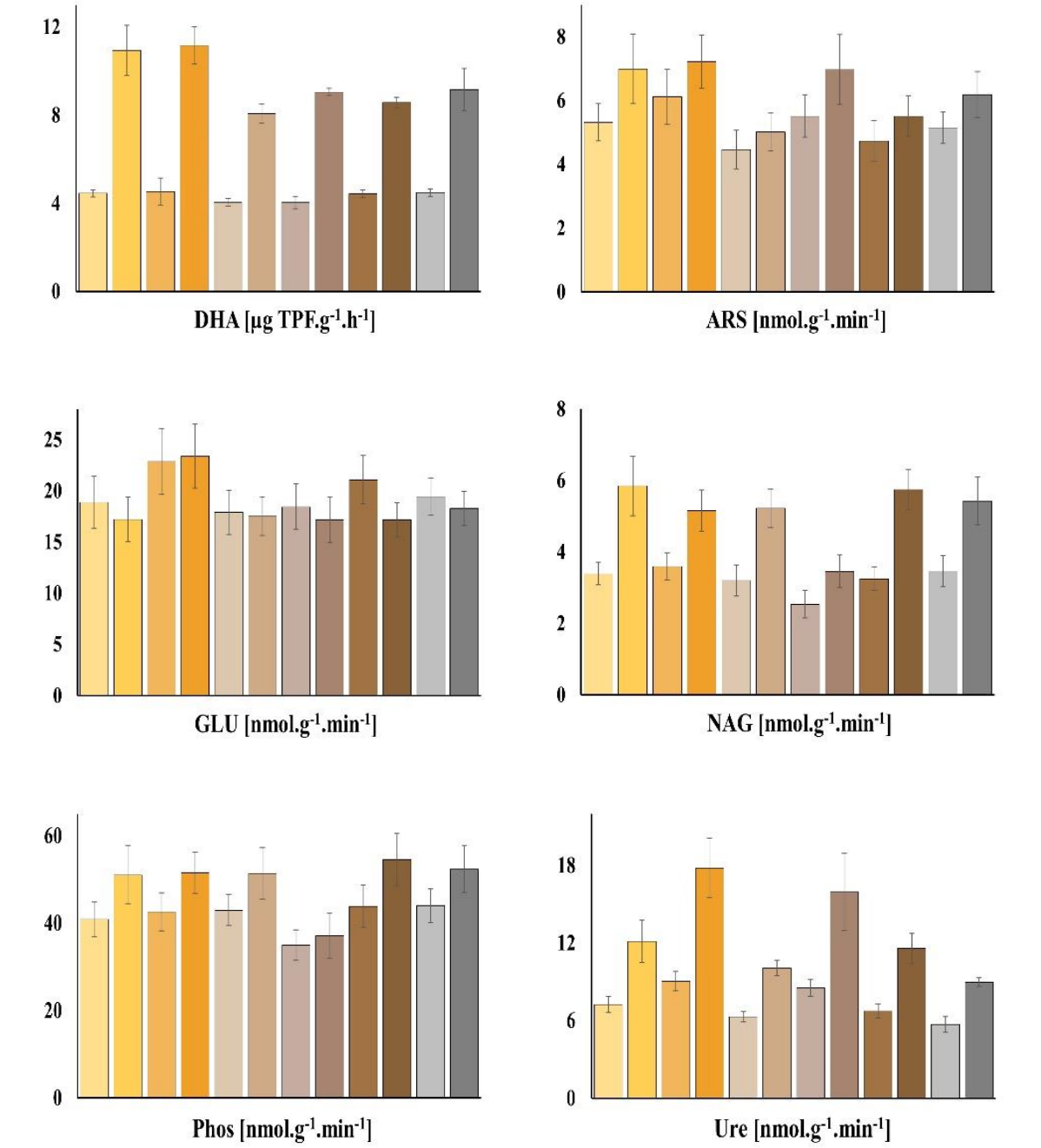

Fig. 12: Comparative Analysis of Enzyme Activities in Soil Amended with P3HB and Other Soil Amendments with or without P3HB. Average values of independent replicates (n = 3), error bars = standard deviation.

DHA

Enzyme dehydrogenase (DHA) is a key determinant of soil microbial activity. DHA activity was considerably higher (an almost two-fold increase) in samples amended with P3HB (Fig. 12). The most significant differences were observed in soil with P3HB only or post-applied to the compost. In these samples, the activity increased by 145 %. The effect of organic fertilizers was relatively neutral without the presence of P3HB. The slightest difference was observed in samples with PHB + manure.

The activity of DHA is responsible for microbial redox processes within the soil system, as well as the oxidation of SOM (Gu et al., 2009). Moreover, it is considered an indicator of soil fertility (Wolinska & Stepniewsk, 2012). DHA represents the potential for carbon mineralization. The study's findings reveal that P3HB-amended soils displayed similar behaviour, significantly increasing DHA production. This rise in DHA could be attributed to the higher proportion of easily mineralizable carbon present in the soil. This is in accordance with Brtnicky et al. (2022), who have also suggested that including PHB in the soil serves as a source of energy or carbon, accelerating the soil's degradation rate.

ARS

The results from determining the activity of arylsulfatase indicated the enhancement of ARS activity by P3HB-amended soils in all experimental variants (Fig. 12). Notably, the most significant increase of ARS activity was detected in the sample with the P3HB alone and in a combination of P3HB with compost or biochar 45 t/ha.

The enzyme ARS is involved in the S mineralization, and its activity is correlated with soil microbial biomass and the rate of S immobilization (Vong et al., 2003), pH and SOC (Goux et al., 2012). Previous research indicates that an increase in ARS activity is usually associated with an increase in these factors. This suggests that P3HB could potentially improve soil fertility and nutrient cycling by stimulating sulfur immobilization.

GLU

The β-glucosidase activity (GLU) was substantially enhanced by the combination of P3HB + compost (Fig. 12). However, in other cases, the impact of P3HB was relatively low compared to the other amendments applied independently. Moreover, the sole implementation of P3HB into the soil matrix led to a decline in the activity of GLU, thereby indicating a negative effect. The enhancement of GLU activity would maintain the degradation of complex carbohydrates (cellulose, hemicellulose) to be used as a source of energy by the microorganism.

GLU is an enzyme that plays a role in a carbon cycle by catalysing the final step in cellulose degradation, producing glucose, a crucial energy source for soil microorganisms. GLU activity is an essential indicator of soil quality and can be used to determine the level of SOM degradation and soil carbon utilization (Stege et al., 2010; Stott et al., 2010). The increase in GLU activity observed in the study indicates that P3HB application triggered the degradation of cellulose. The highest GLU activity was observed in compost-amended soil, which could be due to the high cellulose content in the compost compared to other amendments used. This increase in GLU activity indicates healthy soil, while low levels could suggest poor soil quality.

Similar GLU activity to the control soil was observed in most sample variants, possibly due to P3HB serving as a carbon and energy source over other sources, resulting in reduced GLU activity by mitigating the mineralization of cellulose. Brtnicky et al. (2022) had a similar finding where GLU preferentially degrades P3HB over cellulose, leaving less phosphatase to degrade cellulose. Another possible explanation is that soils with P3HB may have had a high C:N ratio, leading to decreased microbial activity and subsequent reduction in GLU activity. The influence of C:N ratio on the resulting effects also applies to other enzymes, as they are all involved either with carbon or nitrogen mineralization (Brtnicky et al., 2022; Sander, 2019).

NAG and Ure

The enzyme NAG and Ure are both involved in nitrogen cycling. The impact of P3HB on urease and N-acetyl-β-D-glucosaminidase activity was positive, either applied solely or in combination with other amendments (Fig. 12). The most substantial increase was observed with P3HB + compost (Ure) and the sole P3HB. Conversely, the application of organic fertilizers such as biochar, manure, and separate, when used alone, resulted in either neutral or negative effects (specifically biochar and manure) on the enzyme activity. Their reduced activity could have adverse effects on microbial activity, soil fertility, and plant growth and development.

Ure is a crucial extracellular enzyme involved in the initial stages of nitrification by providing a nitrogen source through urea hydrolysis (Roscoe et al., 2000). Nitrification completes the organic nitrogen conversion initiated by ammonification and is vital for soil and ecosystem health in the nitrogen cycle. Hence, urease activity is closely linked to the availability of N in the soil. Enzymes released primarily by soil microorganisms mediate the mineralization of organic nitrogen compounds in soils. Therefore, the increased urease activity observed in the presence of P3HB obtained by our analysis indicates that the P3HB can stimulate the growth and activity of nitrifying microorganisms in the soil, ultimately leading to an increase in nitrogen availability.

As previously mentioned, research indicates that biodegradable plastics, like P3HB, can enhance the soil's water-holding capacity, as shown by de Souza Machado et al. (2018). Studies have demonstrated that urease activity is influenced by the moisture content of the soil. The increase in soil moisture may be a contributing factor to the observed increase in Ure activity. It has been reported that urease activity is affected by soil moisture content (Antil et al., 1993).

Similar to the function of urease has the enzyme NAG, which participates in carbon and nitrogen cycling in the soil (Ekenler & M., 2002). The observed increase in NAG activity of the enzyme in all P3HB-amended soil variants is consistent with the findings of Brtnicky et al. (2022). These results indicate that microorganisms need more nitrogen to decompose P3HB than other organic amendments used in the experiment, comparable to Ure. This suggests a possible correlation between the activity of NAG and Ure, although the enhancement of NAG was more noticeable than Ure. It appears that microorganisms need more nitrogen to decompose higher concentrations of P3HB.

Phos

The experimental study also found that the production of Phos enhanced similarly when treated with a single amendment of P3HB or in combination with other amendments (except for P3HB with manure), as shown in Fig. 12. The co-addition of P3HB and manure demonstrated the most significant enhancement of Phos activity. The amendments, such as compost, manure, and separately present solely in the soil, did not result in changes in the microbial activity of Phos. In contrast, the biochar 45 t/ha decreased the enzyme activity both when added alone or with P3HB, making the availability of Phosphorus weaker in these soils.

Organic phosphorus in soil is unavailable to plants, so they must mineralise it to access it. Phosphatase helps to hydrolyse organic P for microorganisms and plants, and its production and activity can indicate P availability (Nannipieri et al., 2011). Increased Phos activity after adding P3HB suggests that it influenced microbial activity, and microorganisms started to produce more phosphatase. However, the enhancement of Phos activity was insignificant compared to other analysed enzymes like DHA, Ure or NAG. As in Ure, also the activity of phosphatase is connected to moisture content. This could also be observed from the graphs, where the activity of both enzymes followed a similar trend (except for the difference in the P3HB + biochar variant), although with different rates. To conclude, the impact of the P3HB amendment on phosphorus availability in the soil can range from neutral to positive and may be dependent on the specific combination of other organic fertilizers used.

In conclusion, based on the data, the enzymes ARS, GLU and Phos were found to have relatively low contributions in explaining the effect of P3HB on soil properties. Their inclusion in the analysis did not significantly improve understanding of the relationship between P3HB and soil microbial properties. Therefore, it can be concluded that these variables may not be important for explaining the overall variability in the data.

In contrast, the results indicate that the presence of poly-3-hydroxybutyrate positively impacts the soil properties, as supported by the observed increase in activity of DHA, Urea, and NAG in soil samples amended with P3HB. P3HB is a storage compound produced by diverse microorganisms in response to nitrogen deficiency and stress (Zhou et al., 2021), which may explain the observed increase in microbial activity. This suggests the positive effect of the biopolymer on nitrogen cycling. What should be highlighted is that all results on the enzyme activity are strongly influenced by soil type, as well as the abundance and biodiversity of the microbial community. This may explain the differences in results observed across the current scientific literature.

4.2.2 Soil respiration assays

This section will explore the effect of P3HB on the soil when applied alone or in combination with other organic fertilizers. The soil microbial activity was assessed by determining basal respiration (determined through CO_2 production rate) and substrate-induced respiration (determined through CO_2 production rate after substrate addition). Soil respiration is closely linked to organic matter and the composition of the microbial community and is highly influenced by moisture content and porosity in the soil. The BR and SIR were utilized as indicators of the carbon and nitrogen mineralization rate in the soil. The results will be

interpreted based on the graphical illustration of the measured data illustrated in Fig. 13. The mean values of independent replicates (n = 3) are presented with error bars representing the standard deviation.

The application of soil amendments (excluding P3HB) alone generally led to a reduction in soil microbial activity across most variants (Fig. 13). However, there was one exception, Man-SIR in soil amended with manure and biochar at 15 t/ha, where a positive effect on SIR was observed. Additionally, a neutral effect was observed for Tre-SIR following the application of biochar at 15 t/ha and Man-SIR after the incorporation of compost.

The addition of P3HB resulted in a significant increase in both BR and SIR in all experimental samples in contrast to the control sample (Fig. 13). Although P3HB alone showed a noticeable increase, its post-application to other amendments resulted in either a further increase or similar results in most variants. The application of certain amendments (mainly compost, biochar 15 t/ha, separate) alone, except for P3HB, did not significantly affect basal respiration compared to control soil. This implies that the amendment applied alone did not profoundly alter microorganisms in the soil, or the nutrients released during the decomposition of applied organic fertilizers did not adequately stimulate microbial activity.

Notably, there was a significant difference in the post-application of P3HB to biochar at a rate of 15 t/ha and 45 t/ha (Fig. 13). The biochar application at 45 t/ha resulted in a slight decrease, while P3HB in the presence of biochar 15 t/ha was noted by a sharp increase in microbial activity. This outcome could be attributed to the high biochar concentration at a rate of 45 t/ha. Therefore, the carbon content was higher, and biochar was possibly more stable and resistant to degradation by the soil microbial community.

The most substantial increase in basal respiration, indicative of increased microbial activity, was observed in the sample treated with P3HB + manure (Fig. 13). Manure is a rich source of organic matter and nutrients, providing a favourable environment for the growth and activity of microorganisms. P3HB, as a biodegradable polymer, served as a source of energy and carbon for the soil microbial community, further stimulating microbial activity.

According to the results, the co-addition of P3HB + biochar (15 t/ha) led to a significant increase in Ala-SIR, Man-SIR, and NAG-SIR levels, indicating a positive effect of this combination on microorganisms' ability to mineralize soil organic matter (Fig. 13). In addition, the highest increases in Pro-SIR and Tre-SIR were observed with the co-addition of P3HB and compost. Furthermore, microbial activity in Glc-SIR and Arg-SIR was enhanced the most by the sole P3HB and then improved significantly by the co-addition of P3HB and biochar 15 t/ha.

Respiration induced by NAG, Arg, Tre and Ala substrates is associated with nitrification and nitrogen mineralization, as these substrates are sources of N (Alkorta et al., 2003; Parham & Deng, 2000). The observed increase in NAG-SIR, Arg-SIR and Tre-SIR in every sample variant (except biochar 45 t/has) implies the priming effect of P3HB on N compounds decomposition, as well as the promotion of nitrogen content in the soil. P3HB, in this case, was proven to potentially benefit soil fertility and plant growth. This correlates with the findings from enzyme activity assays of NAG and Ure.

Glucose substrate is related to soil carbon cycling (Sinsabaugh et al., 2008). Reduced Glc-SIR in biochar, with or without P3HB, suggests stable organic matter formation, which is less available to microbial decomposition and, thus, potentially inhibits microbial activity (Stott et al., 2010). Other used substrates, such as Tre and Man, also contribute to carbon cycling and could help explain the potential carbon limitation of biochar (Osanai et al., 2005). However, the post-application of P3HB to these substrates ultimately increases the respiration rate. This could be due to Mannose and Trehalose being metabolized by different microbes or pathways as glucose, resulting in varying rates and responses to biochar amendment.

The involvement of Ala and Pro in sulfur cycling and their corresponding trends in the resulting graphs indicate a possible association between these two amino acids and the observed changes in arylsulfatase enzyme activity, which is also involved in sulfur cycling (Alkorta et al., 2003; Vong et al., 2003). The increased respiration rate on soil microbial activity by P3HB was highest for the Ala-SIR and Pro-SIR results, which is evident in the corresponding graphs. This could have two possible explanations. Either it suggests that P3HB may positively stimulate the activity of the microorganism responsible for S cycling by providing sources of energy, or it can imply that the content of S in the soil decreased due to the presence of P3HB, as the abundance of sulfur-oxidizing bacteria decreased and was replaced by microorganism favouring P3HB degradation.

Based on the results of the respiration determination, it can be concluded that the incorporation of P3HB is likely to enhance the mineralization of C, N, and P and the overall respiration rate of the soil. This conclusion is supported by the observation that the respiration rate substantially increased in most of the soil samples when P3HB has applied alone or in combination with other amendments. This is likely attributed to the high content of SOM in the soil and enhanced microbial activity. The positive effect of P3HB on microbial activity was also revealed in previous enzyme activity assay (Song et al., 2014; Zheng et al., 2009).

The obtained results contradict the findings of previous studies examining the impact of conventional microplastics soil health properties. Specifically, the studies indicate that microplastics can inhibit soil enzyme activity and reduce soil respiration (Zang et al., 2020; Zhao et al., 2021; Zhou et al., 2021). The results of this thesis demonstrate the potential of biodegradable plastics, specifically P3HB, to mitigate the negative impacts of still widely used conventional plastics on soil health. Additionally, biodegradable P3HB may enhance specific soil properties and further improve soil health. Therefore, the transition towards the use of biodegradable plastics is suggested to address the negative impact of conventional plastics on the soil.

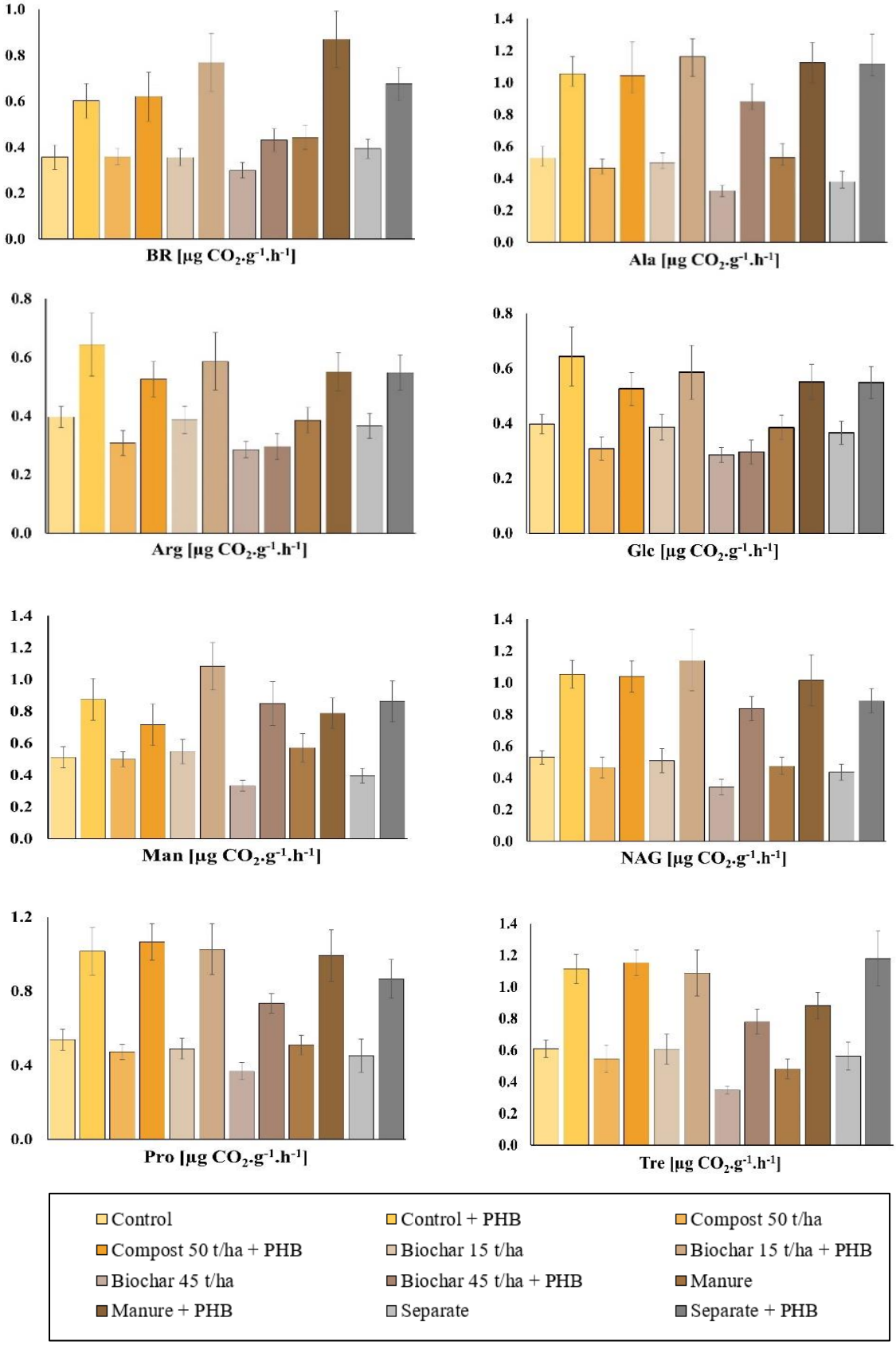

Fig. 13: Comparative analysis of soil respiration in response to P3HB and other soil amendments, including compost, biochar, manure, and separate. Average values of independent replicates (n = 3), error bars = standard deviation.

4.3 Thermal analysis of P3HB in the soil

Thermal analysis, i.e. thermogravimetry, was conducted in this study to investigate the thermal properties of the soil, the rate degradation of poly-3-hydroxybutyrate and the potential impact of co-amendments, such as compost, manure, separate and biochar, on the P3HB degradation. As the temperature during the experiment increases, the compounds present in the soil undergo thermally induced transformations resulting in a mass loss, while others remain intact. The soil samples can be assessed for the degradation of volatile fractions, thermally unstable and stable fractions of organic matter, and minerals. The mass loss occurs in multiple stages, represented as intervals (temperature zones), where each interval corresponds to the mass loss of a specific fraction. The mean thermal mass losses (TML) for each sample were calculated using the data obtained in the temperature range of 20 to 700 °C and by dividing them into 10 °C temperature intervals. In this work, e.g. TML_{100} refers to mass loss in the interval 90–100°C, and, e.g. $TML_{200–300}$ refers to mass loss between 200 and 300°C. This step is aimed at minimizing the dataset while maintaining the reproducibility of the results (Kučerík et al., 2018).

The particular intervals have been distinguished by different colours in Fig. 14. They serve for a better understanding of the ongoing processes and assessment of the whole degradation process. The exact values of temperature intervals may slightly differ within the samples as they are influenced by the soil's chemical composition and properties. However, as the soil used for the experiments in this thesis did not differ within the sample, they can be used for comparison of changes induced by addition of amendments and biodegradation P3HB.

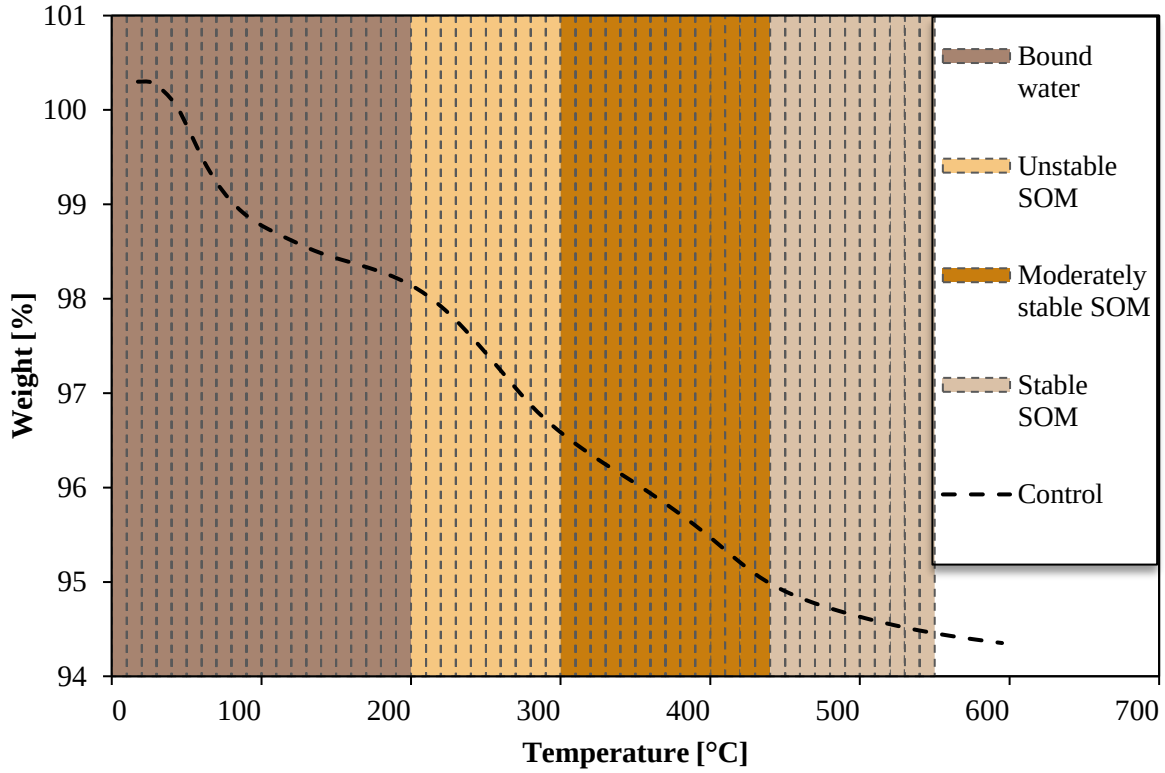

Fig. 14: The temperature interval associated with the decomposition of specific compounds.

The temperature zones from Fig. 14 representing changes (losses) within the soil are distinguished as follows:

- 30–100 °C loosely bound water, particularly on SOM;
- 100–200 °C strongly bound water on mineral surfaces;
- 200–300 °C degradation of unstable SOM;
- 300–450 °C moderately stable SOM;
- 450–550 °C stable SOM;
- > 550 °C degradation of carbonates (Kučerík et al., 2018).

The obtained records reflect the alterations in soil characteristics resulting from the addition of P3HB. Moreover, the records that display the combined application of P3HB with other soil amendments (such as compost, biochar, manure and separate) demonstrate that the presence of P3HB and its subsequent biodegradation resulted in a more significant deviation in the TG curve compared to the situation when the amendments were applied individually. In fact, any differences from the control sample indicate changes in the soil properties.

The data obtained from the thermal analysis of samples with the addition of compost and P3HB to the soil showed a mass loss trend that was comparable to blank samples with the addition of P3HB alone, with only a marginal reduction in mass loss. Within up to 100°C, the application of compost to P3HB resulted in the release of more loosely bound water compared to the other samples or the P3HB presence alone. These results are also apparent in the P3HB + compost TG curve (Fig. 16), where the impact of compost on P3HB degradation was minimal, and the compost curve alone is nearly identical to the blank.

Conversely, a completely opposite trend was observed in the samples containing 15 t/ha of biochar, where it was a noticeable decrease (i.e. lower mass loss) in both the decomposition of stable organic matter ($TML_{450–550}$) and the release of water ($TML_{20–200}$) during the thermogravimetric analysis. The other amendments also resulted in a decrease in mass loss, although the effect was less pronounced than that of biochar 15 t/ha. In the temperature zone 200–300 °C (TML_{300}), the mass loss examined in all samples containing P3HB was similar. Within this range, the mass loss of soil organic matter was estimated to be approximately twice as high as that of the blank sample. The order of effectiveness in mitigating degradation of organic matter, from most effective to least effective, is as follows: biochar at a rate of 15 t/ha (Fig. 17), manure (Fig. 19), biochar at a rate of 45 t/ha (Fig. 18), separate (Fig. 20), and compost (Fig. 16).

In Fig. 15–Fig. 20, each sample displays a mass loss that extends up to 100 °C or 200°C, which represents the release of loosely or chemically bound water. This is followed by the release of water that is firmly attached to the surfaces of organomineral complexes or SOM pores in the temperature interval 100–200 °C ($TML_{100-200}$). This is due to residual water content resulting from air drying and exposing the soil to 43% RH prior to the analysis. All samples follow a similar trend within this temperature range with only slight deviations, suggesting that the water retention capacity of the soil was not significantly affected by the application of soil amendments.

On the contrary, as already mentioned, the application of the biochar 15 t/ha resulted in the most significant reduction in water loss during thermal analysis. This could be explained by the high porosity (and specific surface area) of biochar, which allows for water molecules to be held in small pores. Consequently, during heating, more energy is required to release the water compared to when the molecules are adsorbed onto soil particles. Therefore, the use of biochar can improve the water-holding capacity of the soil (Adhikari et al., 2022). Notably, the presence of P3HB did not influence this outcome, even in the post-application to compost, manure, or biochar 45 t/ha, slightly decreased mass loss in the area. Overall, it is theoretically possible that the increased organic matter content resulting from the use of some fertilizers could improve soil water retention, potentially leading to decreased mass loss in the area.

The primary decomposition can be observed from the temperature range of 230–300 °C, as shown in Fig. 15. This temperature range is associated with the decomposition of labile organic matter, and it is also the temperature interval under which P3HB, as a biodegradable polymer, undergoes degradation (Fojt et al., 2022). Therefore, the P3HB-amended soil samples exhibit a more significant break in the TG curve than the control. The measured values of $TML_{200–300}$ were estimated to be approximately twice as high as that of the blank sample, indicating that the P3HB was not completely decomposed yet. This finding suggests that the residues of P3HB may still have a potential impact on the soil, as suggested by the work of Fojt et al. (2022).

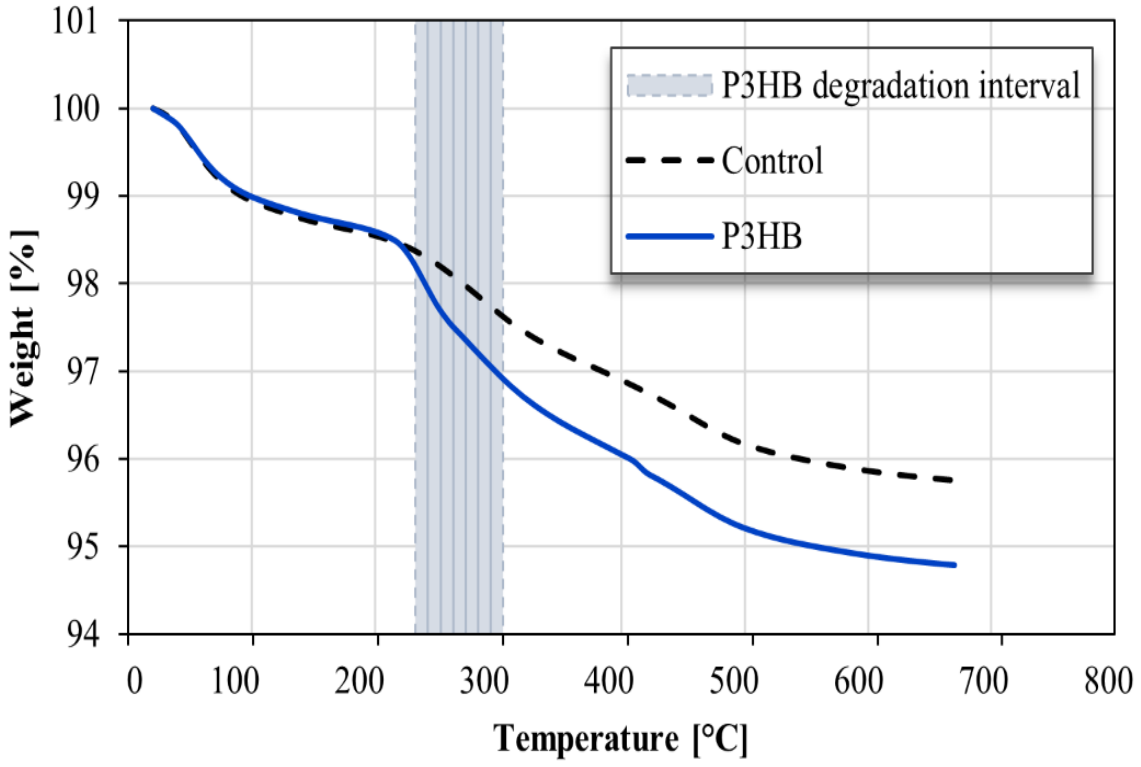

Fig. 15: Mass loss of P3HB-amended soil as a function of temperature.

Following the break observed at 230–300 °C, there is a gradual mass loss until the analysis reaches a constant temperature of 680 °C. Compared to a control sample, the presence of P3HB contributed to a substantial increase within the interval of 300–450 °C. The degradation

products of P3HB that started to release from 230 °C might serve as a source of energy for microorganisms which increased their activity, ultimately resulting in higher biomass production.

The interval of 450–550 °C is associated with the degradation of persistent organic matter and minerals in the soil. The P3HB-amended soil did not result in a higher decomposition rate, and the mass loss was almost comparable to blank. This confirms that P3HB was already degraded in the previous temperature zone, and only stable organic matter induced mass loss in this temperature zone. Within this zone, the biochar 15 t/ha contribution to the soil was most noticeable, as its presence substantially decreased the mass loss in comparison to the control soil. This trend continued until the analysis reached a constant temperature of 680 °C.

The study found that the presence of organic amendments in soil affected the degradation process of P3HB, as demonstrated by the variation in the mass loss of SOM in soil samples containing P3HB and organic amendments (Fig. 16–Fig. 20) compared to the control sample with P3HB only (Fig. 15). To evaluate the extent of this effect, the residual P3HB in each soil was quantified. This was achieved by calculating the difference in mass loss between soil samples co-amended with both the amendment and P3HB and those with only the control amendment. For instance, to quantify the amount of residual P3HB in compost-amended soil, the mass loss from a soil sample containing P3HB and compost is subtracted from the mass loss in a sample containing compost only.

The total amount of P3HB remaining after the thermogravimetric analysis within a 200–300 °C temperature interval (i.e. the amount of biopolymer that was not oxidised) for each sample is reported in Tab. 4, with a lower residue indicating a more rapid degradation of P3HB due to the presence of other soil amendments. The standard deviation for thermogravimetric analysis was determined by calculating the mean of three repetitive measurements of both non-amended and amended samples in the selected temperature interval.

Tab. 4: The residual P3HB remaining in the soil after P3HB decomposition within the temperature interval 200–300 °C.

$TML_{200\text{-}300}$	***Residual P3HB [%]**
Control soil + P3HB	0.73 ± 0.06
Compost 50 t/ha + PHB	0.67 ± 0.06
Biochar 15 t/ha + PHB	0.66 ± 0.03
Biochar 45 t/ha + PHB	0.69 ± 0.06
Manure + PHB	0.63 ± 0.06
Separate + PHB	0.66 ± 0.08

* Values calculated as average from independent replicates (n = 3) ± standard deviation.

The residual P3HB present in the soil was 0.73% under the sole application. However, the addition of compost, biochar (15 t/ha and 45 t/ha), manure, and separate resulted in residual P3HB of 0.67%, 0.66%, 0.69%, and 0.63%, respectively. These results show that the incorporation of organic amendments decreases the amount of residual P3HB in all sample

variants, comparing the values to control samples. It can be concluded that the degradation of poly-3-hydroxybutyrate was supported by all applied amendments, with the most significant effect observed following the application of manure.

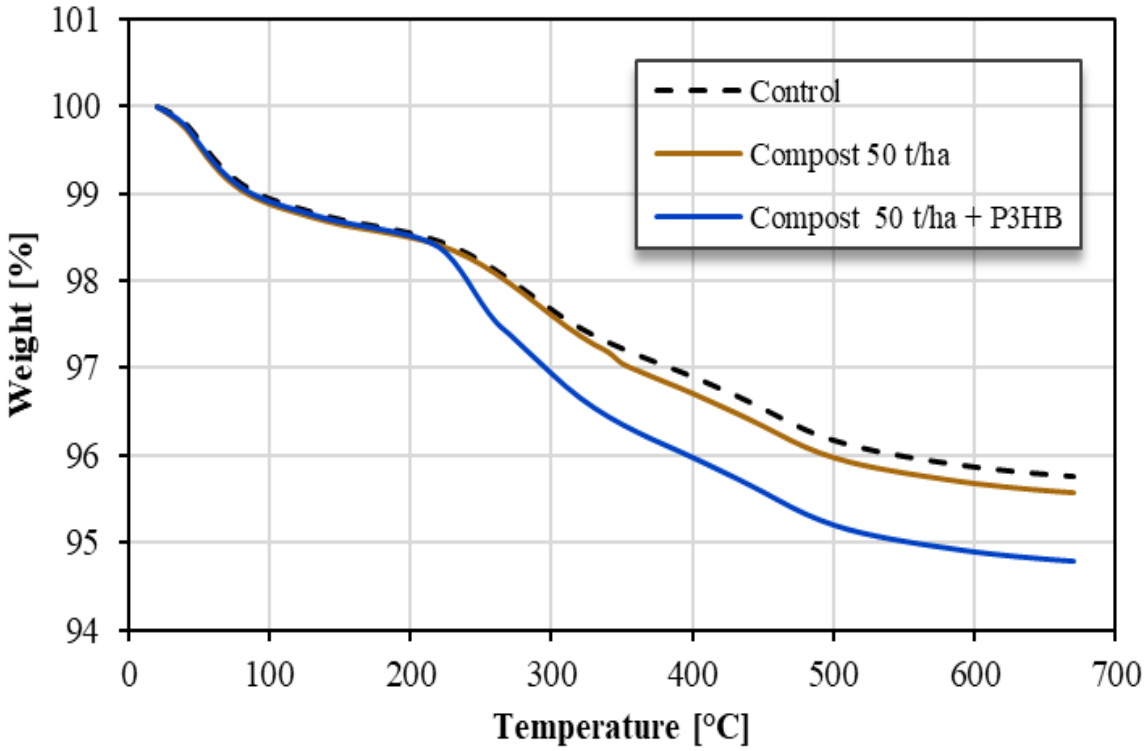

Fig. 16: Mass loss of soil with a post-application of P3HB to compost as a function of temperature.

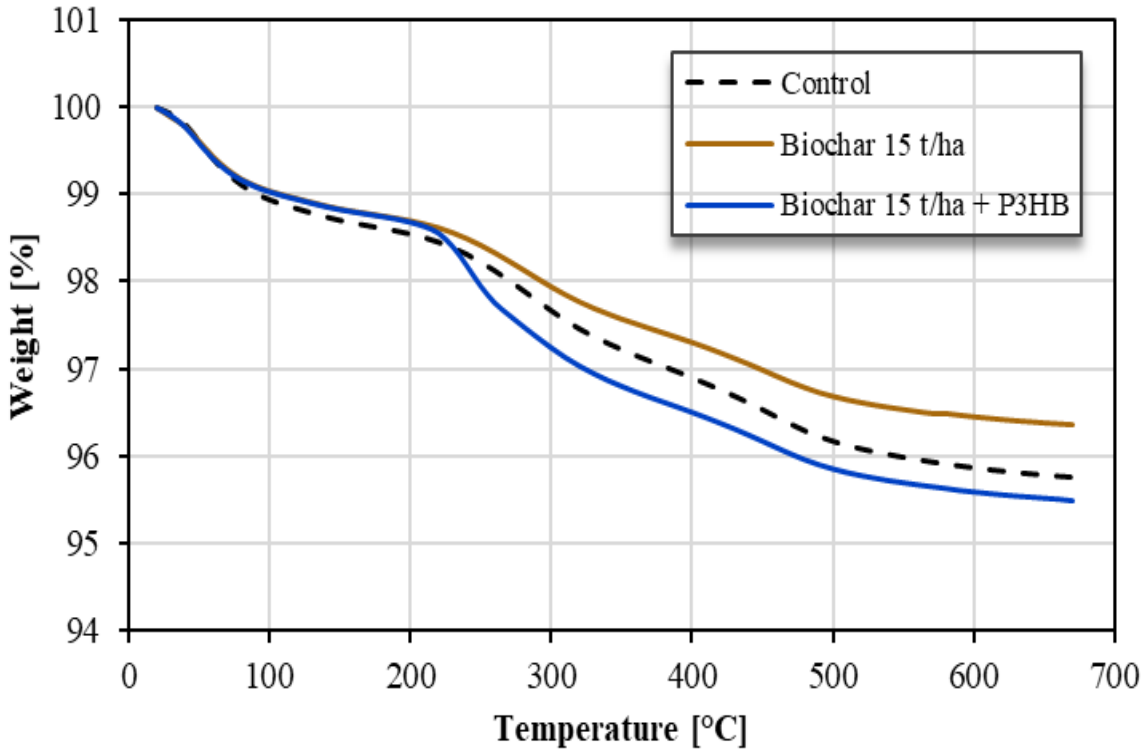

Fig. 17: Mass loss of soil with a post-application of P3HB to biochar 15 t/ha as a function of temperature.

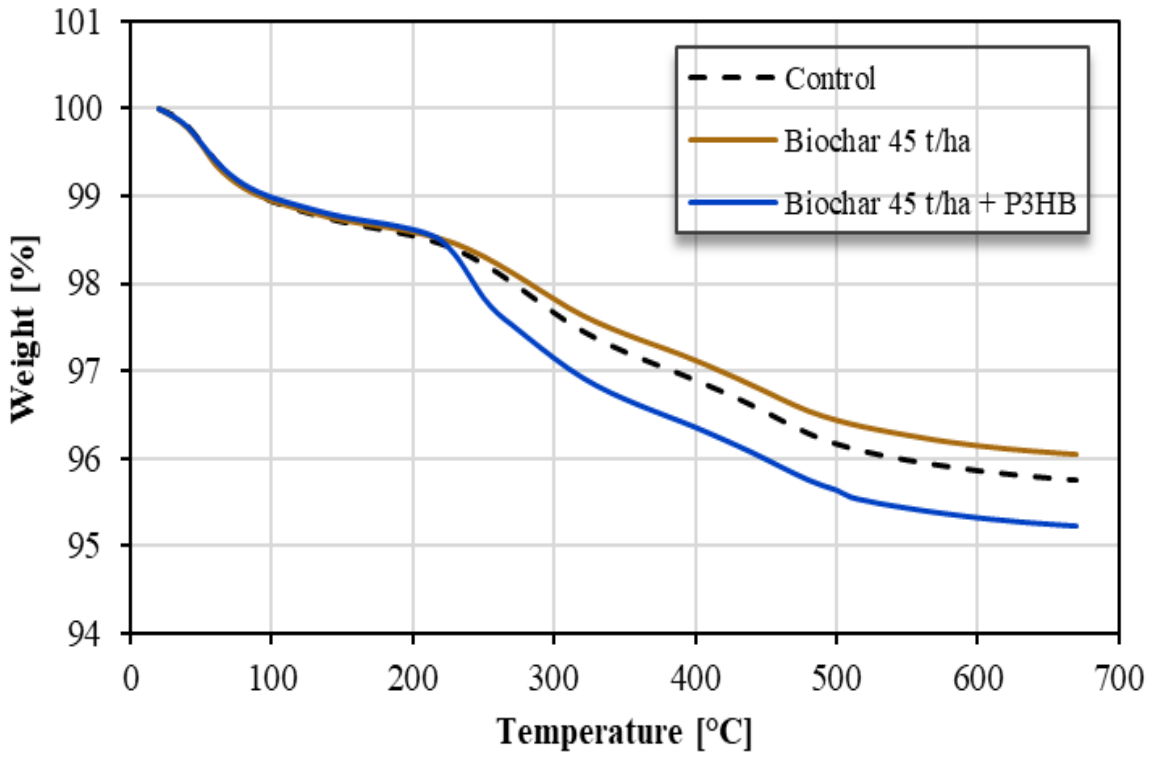

Fig. 18: Mass loss of soil with a post-application of P3HB to biochar 45 t/ha as a function of temperature.

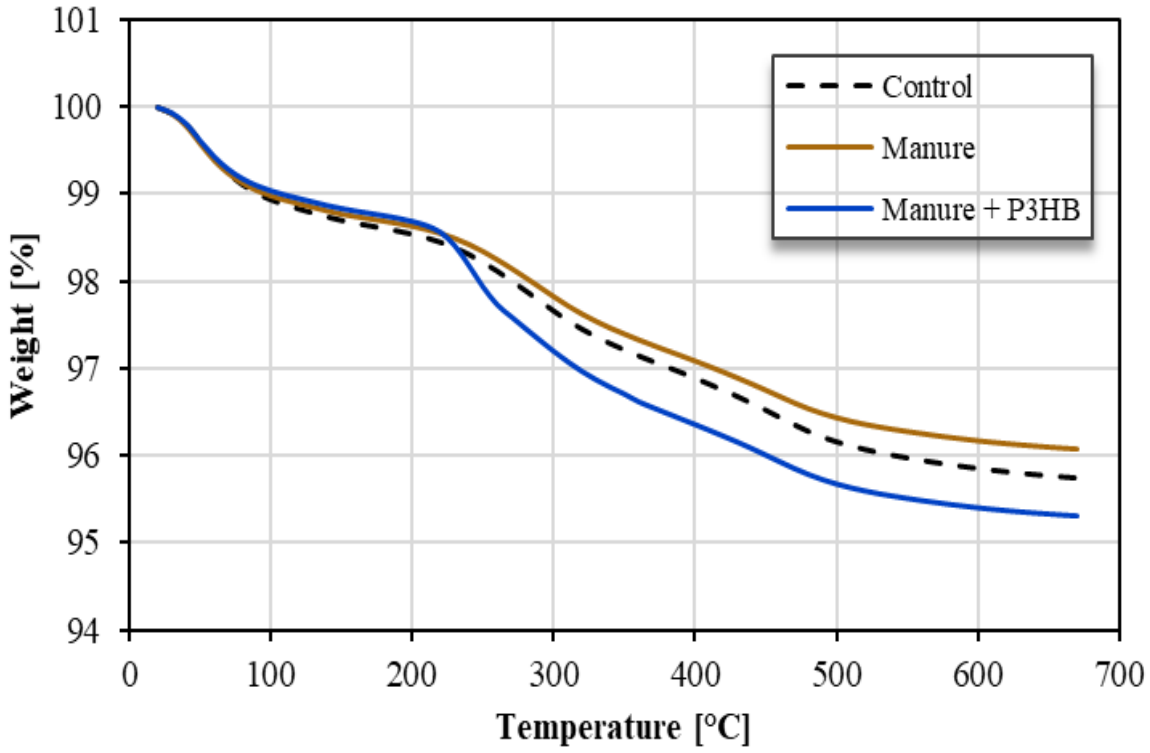

Fig. 19: Mass loss of soil with a post-application of P3HB to manure as a function of temperature.

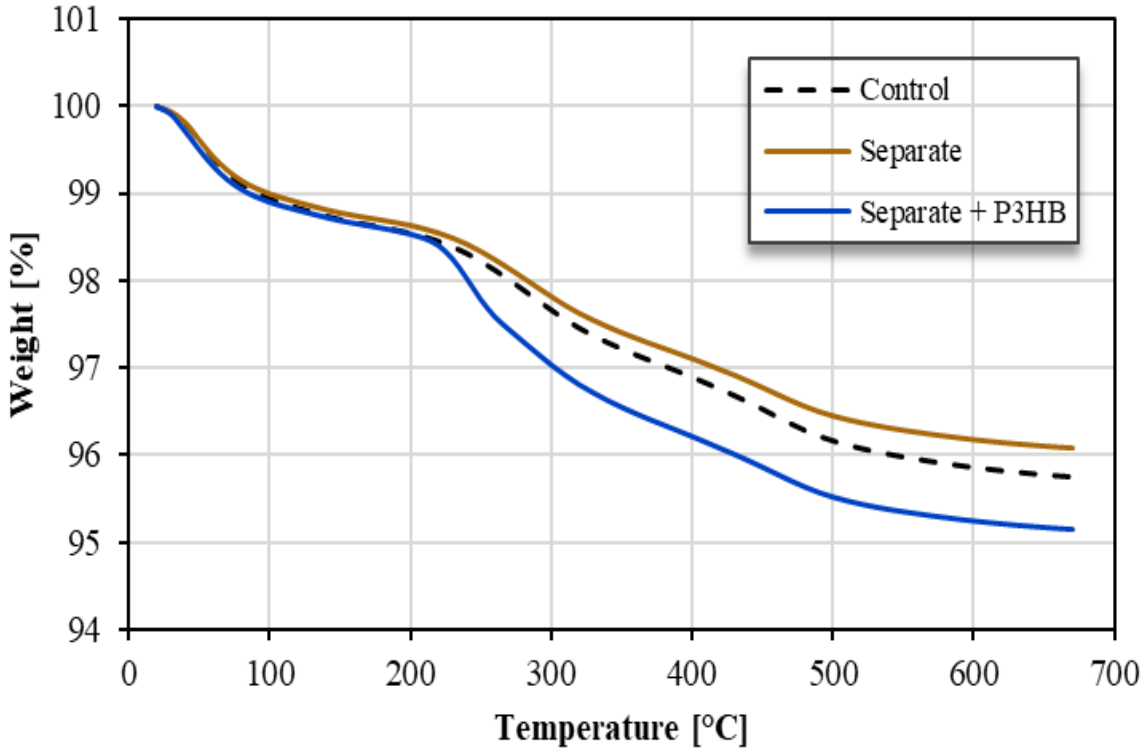

Fig. 20: Mass loss of soil with a post-application of P3HB to separate as a function of temperature.

5 CONCLUSION

This study investigated how the biodegradable polymer, poly-3-hydroxybutyrate, influences various physical, chemical, and biological soil properties that are essential for soil quality and agricultural productivity. To achieve the study's objectives, the plant aboveground biomass, pH, dry matter content, enzymatic and substrate-induced respiration, and thermogravimetry were analysed. These parameters allowed for a comprehensive evaluation of the impact of P3HB on soil quality, providing valuable insights into its potential as an agricultural tool.

As anticipated, the applied P3HB to soil addition had a pronounced and similar negative impact on the dry aboveground biomass of maize plants. Application of P3HB on soil previously amended with organic amendments (compost, biochar, manure and separate) did not improve this situation much. P3HB slightly reduced the soil dry matter, but dry matter was enhanced for all combinations of P3HB with organic amendments, except compost. Furthermore, solely applied P3HB increased the microbial activity of all studied enzymes and soil microbial respiration. Its effect was amplified substantially with compost in the case of enzymes and biochar 15 t/ha in terms of SIRs.

Moreover, no significant alteration in soil pH has been observed, although there was a slight reduction. However, the adverse effects of P3HB on dry matter content and aboveground mass may have more significant implications for plant growth and productivity compared to the positive effects on soil enzyme activity and microbial respiration. The study results suggest that the post-application of P3HB to organic amendments can mitigate these adverse effects of P3HB. Notably, using P3HB in combination with biochar at a rate of 15 t/ha was particularly successful, as observed across multiple assessed factors. The hypothesis that the addition of soil amendments promotes the degradation of P3HB, resulting in a decreased amount of residual polymer in the soil, was confirmed by the results obtained from the thermogravimetric analysis. A reduction in the amount of polymer could potentially mitigate its impact on the soil.

While the study's findings suggest that applying P3HB after the use of organic amendments, particularly biochar and compost, could slightly mitigate the adverse effects of P3HB, further research is needed to optimize the application rates of P3HB and identify the most effective amendment to be used in combination. It is crucial to consider different soil types, crops, and environmental conditions when designing future studies to ensure the results are robust and applicable in diverse agricultural settings.

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LIST OF ABBREVIATIONS AND SYMBOLS

3-HB 3-hydroxybutyrate

AGB aboveground biomass

Al aluminium

Ala L-alanine

ARS arylsulfatase

ATP adenosine triphosphate

C carbon

Ca calcium

CEC cation-exchange capacity

CH_4 methane

CoA coenzyme A

CO_2 carbon dioxide

DDS drug delivery systems

DOM dissolved organic matter

EC electrical conductivity

Fe iron

Glc D-glucose

GLU β-Glucosidase

HDPE high density polyethylene

ISO international standardisation organisation

K potassium

LDPE low density polyethylene

Man D-mannose

Mg magnesium

Mn manganese

MO microorganisms

N nitrogen

N_2O nitrous oxide

NAD nicotinamide adenine dinucleotide

NAG N-acetyl-β-D-glucosamidase

O_2 oxygen

OM organic matter

P phosphorus

PBAT polybutylene adipate terephthalate

PBS polybutylene succinate

PBSA poly(butylene succinate-co-butylene adipate)

PCL polycaprolactone

P3HB poly-3-hydroxybutyrate

PE polyethylene

PET polyethylene terephthalate

PGA poly(glycolic acid)

PHA polyhydroxyalkanoates

PHBV poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

Phos phosphatase

PLA polylactic acid

PLGA poly(lactic-co-glycolic acid)

PP polypropylene

Pro protocatechuic acid

PVC polyvinyl chloride

S sulphur

Si silicon

SIR substrate-induced respiration

SOC soil organic carbon

SOM soil organic matter

TCA tricarboxylic acid cycle

TG thermogravimetry

TGA thermogravimetric analysis

TN total nitrogen

TPF triphenylformazan

TTC triphenyltetrazolium chloride

Tre D-trehalose

Ure urease

Zn zinc