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Long-term effects of repeated inputs of organic wastes in agriculture on crop production and soil fertility: insights from two French long term field experiments

Effets à long terme d'apports répétés de produits résiduaux organiques en agriculture sur la production agricole et la fertilité des sols : apports des résultats de deux essais au champ de longue durée en France

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Graduate School : Biosphera. Référent : AgroParisTech

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Mots clés : Produits résiduaux organiques; Production agricole; Impacts environnementaux; Fertilité du sol ; Santé du sol

Résumé : Les engrais minéraux jouent un rôle crucial pour assurer la sécurité alimentaire mondiale. Cependant, la production de ces engrais est fortement tributaire de ressources non renouvelables, et leur utilisation excessive peut entraîner divers problèmes environnementaux et sanitaires. Le recyclage des produits résiduaux organiques (PRO) en agriculture peut améliorer la fertilité des sols et substituer les engrais minéraux. Ce travail de thèse vise à évaluer les impacts à long terme d'applications répétées de PRO en agriculture sur la production agricole et la fertilité du sol, ainsi que les déterminants de ces effets. À cette fin, nous avons mobilisé les résultats de deux essais au champ de longue durée situés en France et qui testent différents PRO représentatifs de ceux fréquemment utilisés en zone périurbaine (composts urbains, fumier bovin, boue d'épuration), dans deux conditions de sol, climat et système de culture différentes. Plus précisément, nous avons étudié i) la dynamique des rendements des cultures et des propriétés des sols, ii) les facteurs déterminants des changements dans les rendements des cultures et les propriétés des sols, et iii) les différences d'effet entre les différents types de PRO et les sites.

Dans l'ensemble, notre étude a montré les avantages et les limites potentiels de l'utilisation de PRO en agriculture. Les applications à long terme de PRO améliorent principalement la fertilité chimique du sol, sans avoir d'impact négatif sur l'activité biologique du sol. L'amélioration des propriétés du sol par les PRO peut être limitée dans les sols qui sont déjà très fertiles. Une substitution partielle des engrais minéraux par les PRO est possible sans diminuer les rendements. Cependant, l'application de PRO seuls aux doses réglementaires européennes peut ne pas être suffisante pour atteindre des rendements optimaux. De plus, l'influence de l'application de PRO sur les propriétés du sol et le rendement des cultures dépend fortement de plusieurs facteurs, notamment des flux d'apport de carbone et de nutriments dépendant eux-mêmes du type de PRO et de la dose d'application, et des propriétés initiales du sol. Néanmoins, l'intégration du recyclage des PRO dans un plus large éventail de pratiques agricoles durables peut constituer une stratégie judicieuse pour renforcer la fertilité des sols et améliorer la productivité des cultures.

Title : Long-term effects of repeated inputs of organic waste in agriculture on crop production and soil fertility: insights from two French long term field experiments.

Keywords : Organic waste; Agricultural production; Environmental impacts; Soil fertility; Soil health

Abstract : Fertilizers play a crucial role in ensuring global food security. However, mineral fertilizer production relies on non-renewable resources, and their excessive use result in environmental and health issues. Recycling organic waste (OW) in agriculture can improve soil fertility and substitute mineral fertilizers. This work aims to assess the long-term effects of repeated applications of OW in agriculture and their driving factors. We used two French long-term field experiments testing various OW representative of frequently used OW in periurban agriculture (urban composts, farmyard manure and sewage sludge) with two different cropping system, soil and climate conditions. Specifically, we investigated i) the dynamics of crop yields and soil properties, ii) the driving factors behind changes in crop yields and soil properties, and iii) the differences of the effects between the different types of OW and the two sites.

Overall, our study has shown the potential benefits and limitations of using OW in agriculture. Long-term applications of OW mainly improve soil chemical fertility without negatively impacting soil biological activity. The extent to which OW can improve soil properties may be constrained in soils that are already fertile. A partial substitution of mineral fertilizers is possible without decreasing crop yields. However, applying OW alone at European regulatory rates may not be sufficient for achieving optimal yields. Additionally, the influence of OW application on soil properties and crop yield heavily depends on several factors, especially on the input fluxes of carbon and nutrients related to the type of OW and to the application rate, and on the initial soil properties. Nevertheless, integrating OW recycling into a wider range of sustainable agricultural practices can be a judicious strategy for enhancing soil fertility and improving crop productivity.

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LIST OF ABBREVIATIONS

AK: Available potassium

ANOVA: Analysis of variance

ANR: Apparent N recovery

AP: Available phosphorous

ARN: Arylamidase

ARS: Arylsulfatase

BFN: Bacterial feeder Nematodes

BIO: Biowaste compost

CEC: Cation exchange capacity

COL: PROspective experiment

DM: Dry matter

EI: Enrichment index

Evenness.Ba: Evenness of bacteria

Evenness.Fu: Evenness of fungal

FFN: Fungal-feeders Nematodes

FYM: Farmyard manure

FYMC: Farmyard manure compost

GBM: Gradient boosting model

GHGs: Greenhouse gas

GLU: β -glucosidase

GWS: Compost of green waste and urban sewage sludge

IROC: Indicator of residual organic C

LTE/LTFEs: Long-term field experiments

MDS: Minimum data set

MI: Maturity index

MSW: Municipal solid waste compost

MWD.FW: Mean weight diameter determined by the fast wetting

MWD.MB: Mean weight diameter determined by the mechanical breakdown

MWD.WS: Mean weight diameter determined by the wet stirring

MWD: Mean weight diameter

NCR: Nematode channel ratio

NFRV: Nitrogen fertilizer replacement value

OM: Organic matter

OPN: Omnivores-predators Nematodes

OW: Organic wastes

PCA: Principal component analysis

PFN: Plant-feeder Nematodes

PHOS: Phosphatase

PPI: plant parasite index

PROC: Potentially residual organic carbon

QUA: Qualiagro experiment

Richness.Ba: Richness of bacteria

Richness.Fu: Richness of fungal

RMSE: Root means square error

RY: Relative crop yield

Shannon.Ba: Shannon index of bacteria

Shannon.Enzy: Shannon index of Enzyme

Shannon.Fu: Shannon index of fungal

Shannon.Nat: Shannon index of Nematodes

SI: Structure index

Simpson.Enzy: Simpson's Diversity Index of Enzyme

SLU: Dehydrated urban sewage sludge

SMB: Soil Microbial Biomass

SMN: Soil mineral nitrogen

SOC: Soil organic carbon

SOM: Soil organic matter

SQI: Soil quality index

TE: Trace elements

URE: Urease

BACKGROUND

Meeting the food demands of a growing population with changing diets necessitates the use of fertilizers, but the production of nitrogen-based fertilizers consumes high amounts of energy, while phosphorus and potassium fertilizers rely on nonrenewable resources. Additionally, excessive use of chemical fertilizers can lead to various environmental and health issues. Therefore, finding alternative methods to maintain crop yields without relying heavily on chemical fertilizers is essential. Among the many alternatives, recycling of OW is considered one of the most feasible methods. By enhancing soil organic matter content, improving soil physical properties, and increasing the abundance and activity of microbial biomass, OW application can increase crop yields sustainably. However, it is crucial to address the potential negative effects of OW application, such as increased soil heavy metal content. To comprehensively evaluate the impact of OW application, long-term field experiments (LTEs) are the most reliable and relevant method. However, current LTEs have some shortcomings, including the use of excessive OW to accelerate experimental results, limited types of OW used in experiments, and inadequate statistical analysis of experimental data. Therefore, the main objective of this study was to explore the overall impact of OW application on soil using LTEs and to identify the differences in performance among different types of OW, sites, and their driving factors.

Chapter I

General Introduction

1 GENERAL INTRODUCTION

1.1 CROP FERTILIZATION

The world population is expected to grow from today's 7.8 billion to 9.7 billion in the year 2050 (De Wrachien et al., 2021). To meet the demand for food from this growing population and changing diets, food production must increase by 70% by 2050 compared to its current level (FAO, 2009). However, the availability of arable land has not increased with population growth, leading to a decline in global arable land per capita from 0.41 ha in 1961 to 0.25 ha in 2015 (Maja and Ayano, 2021). The development of fertilizers has made possible a rapid increase of food demand with the increased population. The contribution of commercial fertilizers to increasing crop yields is significant, with a conservative estimate of at least a 30%-50% increase (Stewart et al., 2005). Nowadays, approximately 44% of the world's population is fed by N fertilizer synthesis (Erisman et al., 2008).

Fertilizers are typically applied to fields in order to boost crop yields and to ensure that plants receive the nutrients they need to grow and thrive. Their main function is to supply essential nutrients that may be deficient or depleted in the soil, which are crucial for the growth and development of plants. Out of the 17 essential nutrients required for a plant life cycle, carbon (C), hydrogen (H), oxygen (O) can be easily obtained by the plant through air and water, thus their supply to the plant is not an issue (Fageria et al., 2009). However, an adequate supply of the remaining 14 nutrients, including macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S), as well as micronutrients like zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), chlorine (Cl) and nickel (Ni), is crucial for the plant growth and development (Fageria et al., 2009). In terms of plant requirements, N, P, and K are characterized as essential nutrients for plant growth, and are also exported by crops in the largest amounts among all essential nutrients (Aulakh and Malhi, 2005). Thus, N, P, and K fertilizers are the most commonly applied to maximize grain yields in agricultural production (Figure 1-1).

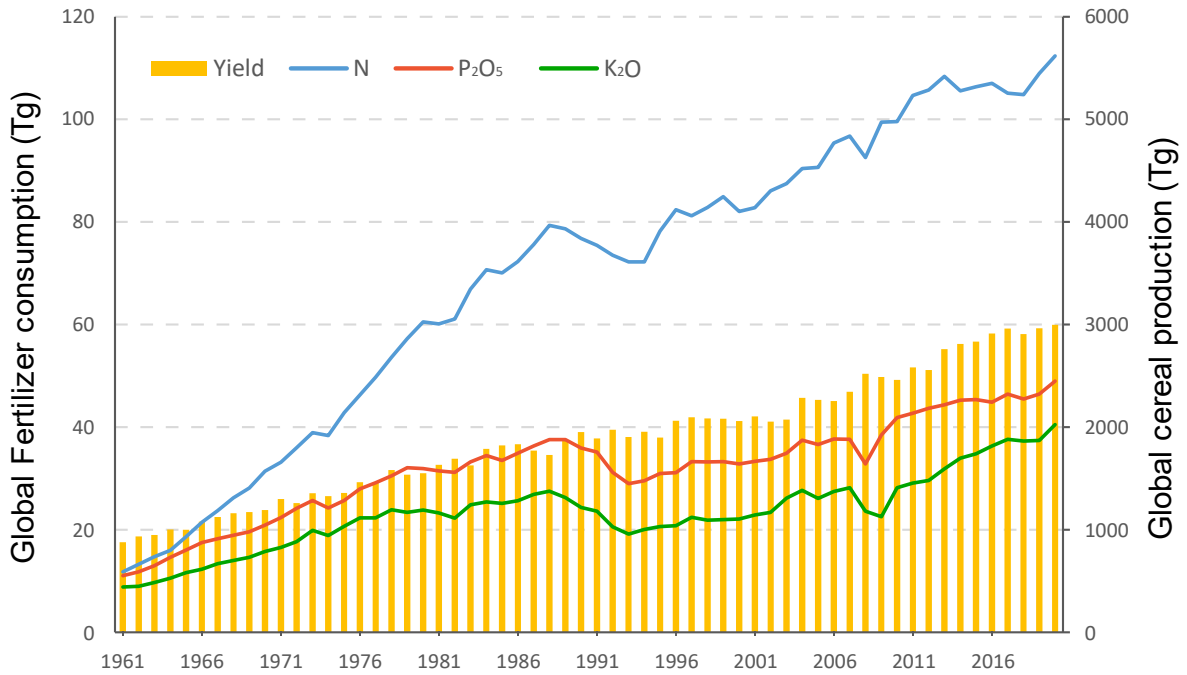


Figure 1-1 Global N, P₂O₅, and K₂O fertilizer consumption and cereal production between 1961 and 2020. (Data source: IFA and FAO).

On the basis of the nutrient supply, fertilizers can be classified into (i) single-nutrient fertilizers and (ii) multi-nutrient or complex fertilizers (Shahena et al., 2021). The more common classification method (Figure 1-2) is based on the source, which can be classified as inorganic fertilizers, organic fertilizers, and biofertilizers (Randive et al., 2021).

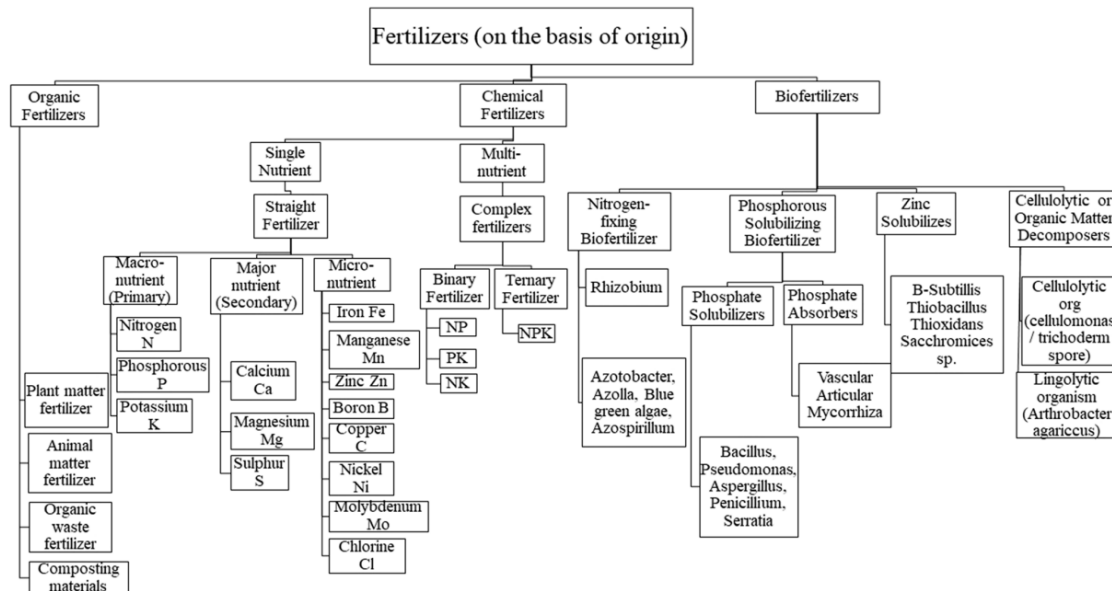


Figure 1-2 Classification of fertilizers. (Randive et al., 2021).

1.1.1 Conventional mineral fertilizer

1.1.1.1 Nitrogen fertilizers

Nitrogen (N), an essential nutrient for plant metabolism, is the most commonly depleted nutrient in soil (Vejan et al., 2021). Insufficient N supply is the primary factor that limits plant growth and productivity (Prasertsak and Fukai, 1997). The Haber-Bosch method, which converts N_2 to ammonia at high pressure and temperature, made it possible to manufacture nitrogen fertilizer industrially (Galloway et al., 2002). The widespread adoption of N fertilizers sustained the continued expansion of the human population, supporting approximately 44% of the world's population through increased food production (Figure 1-3) (Erisman et al., 2008). Nowadays, more than 140 million tonnes of ammonia are produced by the Haber-Bosch process annually, and nearly 80% of them are used as fertilizer in agriculture (Bilgaiyan et al., 2022; Comer et al., 2019). N fertilizers can be classified according to the form as: (i) Nitrate fertilizers; (ii) Ammoniacal Fertilizers; (iii) Ammoniacal-Nitrate fertilizers; (iii) Amide fertilizers (Tiwari et al., 2022). Among the various N fertilizers, urea is the most commonly used because it is rich in N content (46%), and because of its high solubility, and nonpolarity (Prasad, 1998). Numerous experiments have confirmed the positive effects of nitrogen fertilization on crop yields (Lee et al., 2017; Qaswar et al., 2020; Stewart et al., 2005). With the application of potash and phosphate fertilizer, an additional application of N fertilizer can increase yields by 45% (Valkama et al., 2013).

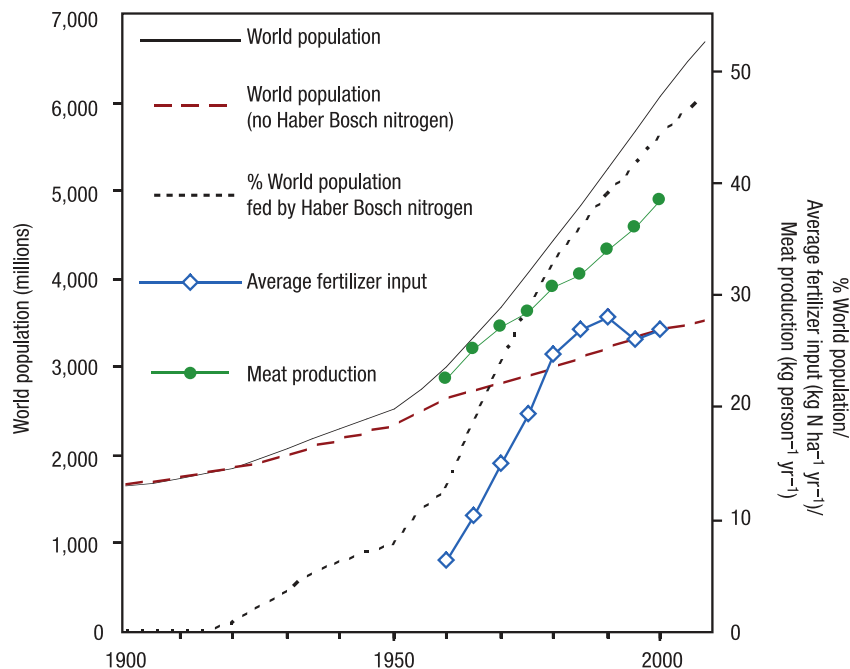


Figure 1-3 Trends in human population and nitrogen use throughout the twentieth century. Of the total world population (solid line), an estimate is made of the number of people that could be sustained without reactive nitrogen from the Haber–Bosch process (long dashed line), also expressed as a percentage of the global population (short dashed line). The recorded increase in average fertilizer use per hectare of agricultural land (blue symbols) and the increase in per capita meat production (green symbols) is also shown.

1.1.1.2 Phosphorus fertilizers

Phosphorus (P) is an essential component of cells and crucial to plant functions such as cell division, energy transfer, and photosynthesis (Balemi and Negisho, 2012). Although phosphorus is the 11th most abundant element in the Earth's crust and quite rich in the soil, most of it is unavailable for plants and unevenly distributed (Cordell and White, 2011). It was reported that more than 30% of the arable land in the world is limited by P availability (Vance et al., 2003). However, it was not until the German chemist Liebig introduced his 'mineral theory' in 1840 that phosphorus became a conventional mineral fertilizer (Cordell and White, 2011). Currently, rock phosphates are the primary source of inorganic P fertilizers and about 90% of global P consumption are used in agriculture, mainly in fertilization (Cordell et al., 2009). To meet food production needs, global P fertilizer consumption has soared from 11 million tons of P_2O_5 per year in 1961 to 48 million tons of P_2O_5 per year in 2020 (Figure 1-1). A large amount of input has resulted in a high proportion of the P in the soil coming from anthropogenically mined phosphate rock, estimated to 82% in France for example (Ringeval et al., 2014). Generally, P fertilizer applied alone does not markedly affect crop yield, but with adequate N and K fertilizer, P input can increase yields by 10-20% in different crops and soils (Bindraban et al., 2020). A study reviewed P fertilization trials in Finland and reported that under the N and K fertilizers application, P fertilizers' inputs significantly increase crop yield by 11% (Valkama et al., 2009).

1.1.1.3 Potash fertilizers

Potassium (K) is the most largely found cation in plants and thus involved in all growth-related functions, including respiration, osmoregulation, starch and protein synthesis, enzyme activation, and photosynthate translocation (Vejan et al., 2021). In agricultural production, the two most crucial roles of K are to maintain cellular turgor under osmotic stress and to safeguard photosynthesis in plants (Römheld and Kirkby, 2010). Without sufficient K supply, plants may experience reduced growth, lower yields, and increased susceptibility to stress (Pettigrew, 2008). Moreover, K fertilization could improve N and P use efficiency, thereby increasing crop productivity and quality (Dhillon et al., 2019). Overall, K is the seventh most abundant element in the Earth's crust, comprising approximately 2.6% of the lithosphere (Schroeder, 1979). However, although soil K reserves are large, the majority of croplands worldwide are deficient in K availability (Zörb et al., 2014). K has become a limiting factor in agricultural production systems, requiring the addition of external K inputs in order to mitigate this stress. The predominant K fertilizer used today is potassium chloride (KCl), a naturally mineral extracted from deep deposits which account for 96% of the world's potash production (Prakash and Verma, 2016). In addition, KCl mining produces by-products such as potassium sulfate (K_2SO_4) and potassium nitrate (KNO_3), which are also commercially available and can be used as fertilizer (Zörb et al., 2014). The increase in yield is inextricably linked to the increased use of potash, with a world consumption of 40 million metric tons of potash in 2020, and rising year on year (Figure 1-1).

1.1.2 Challenges of conventional mineral fertilizer

1.1.2.1 Challenges in mineral fertilizer production

Nowadays, the Haber-Bosch process remains one of the most common techniques for the industrial production of ammonia (Suryanto et al., 2019). Although N_2 is an inexhaustible source in the atmosphere, the process is associated with high energy consumption and leads to massive GHGs emission (Comer et al., 2019). It was reported that producing one metric ton of N fertilizer by the Haber-Bosch process requires 873 m^3 of natural gas (Vance, 2001). About 5% of all the natural gas produced in the mid-1990s was used to make ammonia, equivalent to 1.3% of total energy consumption (Jenkinson, 2001). Hoffman et al. (2018) estimated that synthetic N production through the Haber-Bosch process contributed 45–46% of the total energy use and around 20% of the total GHG emissions in conventional grain crop production systems. The heavy dependence on energy also links the cost of nitrogen fertilizers to the fluctuation of natural gas prices, which can have negative impacts on food affordability. For example, the escalation of the Ukraine-Russia war led to an increase in energy prices, which in turn caused a 12% rise in international wheat prices within a three-month period (Alexander et al., 2022).

Unlike N fertilizers, which can be obtained through synthetic, P and K fertilizers are primarily sourced from the mining industry. Currently, approximately 240 million tonnes of phosphate rock (Randive et al., 2021) and 31.5 million tons of potash rock (Prakash and Verma, 2016) are mined annually around the world. A large proportion of the total P and K production in the world is used for making fertilizers, with approximately 80-90% of P production (Hellal et al., 2019) and 90-95% of K production (Jena, 2021) being consumed for this purpose. However, easily mineable deposits of rock are limited. Estimates of the global P reserve could be exhausted within some hundreds of years (Liu et al., 2008; Obersteiner et al., 2013), while the projected life of potash reserves decreased from 351 years in 1996 to around 138 years in 2018 (Al Rawashdeh, 2020). With concerns over reserves as well as increased mining difficulty, the price of raw materials of P and K fertilizers has increased exponentially and led to tremendous pressure on agricultural production (Cordell and White, 2011). Moreover, uneven geographical distribution limits the transport and processing of fertilizers. It was reported that Canada, Russia, and Belarus produce more than 90% of world potash (Ciceri et al., 2015), and Morocco holds nearly 77% of the world's P reserves (Cooper et al., 2011).

1.1.2.2 Challenges in fertilizer application

In addition to the environmental impacts during the manufacturing process (e.g., GHGs emission and water pollution during processing), improper fertilizer application in agriculture can have several other environmental and health effects, including eutrophication of aquatic and terrestrial ecosystems, reduced biodiversity, and altered ecosystem functions (Bleeker et al., 2011). The application of mineral fertilizers has been

shown to directly contribute to the increase in nitrate concentrations in rivers and groundwater (Davies and Sylvester-Bradley, 1995; Ding et al., 2014). Felix and Elliott (2013) compared historical fertilizer application rates with Greenland ice core $\delta^{15}\text{N}$ content in the past two centuries and inferred that increases in twentieth-century commercial fertilizer use led to significant increases in soil nitrogen oxides (NO_x) emissions. Moreover, long-term N fertilization leads to intensified soil acidity (Johnston et al., 1986) while P-fertilizers N fertilization results in higher concentrations of trace metals such as Ni, Cd, Zn, Pb, As, and Cr in the soil (Nziguheba and Smolders, 2008).

The concept of “planetary boundaries” defined as safe operating space for human prosperity concerning the Earth system was used to evaluate the magnitudes of human impacts on earth-system processes (Rockström et al., 2009). Boundaries are based primarily on climate change and the integrity of the biosphere, and exceeding these thresholds can lead to dangerous environmental changes in the Earth system (Rockström et al., 2009). On this basis, the proposed planetary boundary for TN and TP are $62\text{-}82 \text{ Tg N yr}^{-1}$ and $6.2\text{-}11.2 \text{ Tg P yr}^{-1}$, respectively (Steffen et al., 2015). However, N and P already transgressed the planetary boundaries in 1970 and 1964, respectively, and are continuing to grow (Sandström et al., 2023). In 2020, the total global application of N and fertilizer were already 112 Tg N yr^{-1} and 20 Tg P yr^{-1} , respectively (Figure 1-1).

1.1.3 Mineral fertilizers substitute

As mentioned before, the use of traditional mineral fertilizers is facing several challenges and it is essential to reduce their usage and explore suitable alternative options. With effective planning and implementation of sustainable practices, the food system has the potential to provide healthy and sustainable diet for a projected global population of 10 billion by the year 2050 (Willett et al., 2019). On one hand we can choose to reduce meat consumption and food waste to reduce the need for mineral fertilization and improve fertilizer use efficiency through precision fertilizer application. It was reported that changing diets towards healthy food, reducing food loss and waste, and improving fertilizer use efficiency and optimizing fertilizer application can reduce N use by 10%, 15%, and 26%, respectively, and P use by 10%, 15%, and 40%, respectively (Willett et al., 2019). On the other hand, we can choose more efficient and environmentally friendly fertilizers to replace traditional ones.

1.1.3.1 Nano-fertilizer

Nano fertilizers are nanomaterials and nano-enabled bulk materials used as fertilizers (Raliya et al., 2018). According to Liu and Lal (2015), nano fertilizers could be classified as (i) macronutrient nano fertilizers, which comprise one or more macronutrient elements (e.g., N, P, K, Mg, and Ca); (ii) micronutrient nano fertilizers, which comprise a wide range of essential micronutrients for plants; and (iii) nanomaterial-enhanced fertilizers, which aimed to increase plant-uptake efficiency of the nutrient. In comparison with conventional fertilizers, nano fertilizers significantly increase nutrient

use efficiency due to their high specific surface area and miniature size (Chhipa, 2017). Previous studies have suggested that nano fertilizers can increase crop production by 20-30% compared to conventional fertilizers (Bratovic et al., 2021). Even though nano fertilizers have a higher efficiency rate (50-70%) for the controlled release of nutrients compared to traditional fertilizers (40-50%) (Shalaby et al., 2022), they are still based on fossil resources and therefore do not solve the issue of sustainability.

1.1.3.2 Biofertilizer

Biofertilizers contain living microorganisms that promote plant growth by enhancing the nutrient supply to the host plant (Mahanty et al., 2017). They are used to accelerate microbial activities to improve soil nutrient utilization and protect the plant from pests and diseases (Nosheen et al., 2021). The most commonly used biofertilizers including nitrogen-fixers (N-fixers), potassium solubilizers (K solubilizers), phosphorus solubilizers (P solubilizers), and plant growth-promoting rhizobacteria (PGPR) (Daniel et al., 2022). Biofertilizers have less ecological impact and are highly effective, are able to improve soil fertility and increase crop yield by 10–50% (Nosheen et al., 2021). However, biofertilizers are often viewed as more costly and slower acting on crops than chemical fertilizers, and require special storage consideration (Pal et al., 2015).

1.1.3.3 Slow/controlled release fertilizers

Slow release fertilizers (SRF) are fertilizers containing a plant nutrient in a form that releases or converts to a plant-available form at a slower rate (Fu et al., 2018). The difference between controlled release fertilizers (CRF) and SRF is that CRF usually has a polymer coating for encapsulation (Fertahi et al., 2021). The advantage of SRF/CRF is that the release of nutrients matched to the needs of the plant, the availability of nutrients is maintained, and the risks associated with high concentrations of soluble salts are reduced (Fertahi et al., 2021; Shaviv and Mikkelsen, 1993). In comparison with conventional fertilizers, slow/controlled release fertilizers can increase fertilizer use efficiency by 10-30%, resulting in a 10-40% saving in the amount of fertilizer used to produce the same yield (Hazra, 2014). Despite the environmental and agronomic benefits of SRF/CRF, their practical application in agriculture is still very limited due to their high price (Shaviv and Mikkelsen, 1993).

1.1.3.4 Organic-fertilizer

Organic fertilizers are organic materials that originate from different sources and contain nutrients needed by plants. Natural organic fertilizers are derived from a wide range of organic wastes (e.g., animal manure) and can be processed into commercial organic fertilizers by different harmless treatments (Möller and Schultheiß, 2015). Commercial organic fertilizers are mainly used to fertilize high-value crops such as vegetables and fruits due to their high cost, whereas natural organic fertilizers are less costly and widely used for the fertilization of multiple crops (Möller and Schultheiß, 2015). Organic fertilizers are often considered less efficient and more costly than

industrial fertilizers when it comes to producing food, as they require a larger quantity to achieve the same results and also result in increased transportation and application costs (Cobo et al., 2019). For example, Dong et al. (2020) conducted a study exploring 344 counties in China and reported that the cost of applying organic fertilizer per unit area for corn, vegetables, and apple crops was significantly higher than that of chemical fertilizer. Nevertheless, sustainability and environmental friendliness of organic fertilizers are widely recognized (Jiang et al., 2022). More details about organic fertilizers will be present in next section (section 1.2).

1.2 ORGANIC WASTES (OW)

1.2.1 OW definition and source

So far, there is no definition related to OW in the European legislation. European Environment Agency (EEA) glossary explains the OW as "Waste containing carbon compounds". While the "Waste" is defined as "any substance or object which the holder discards or intends or is required to discard" based on the Waste Framework Directive (75/442/EEC). In general, OW is a biodegradable waste or by-products originating from agriculture, industrial or municipal activities, including animal manures, municipal biosolids, sewage sludge, green waste and so on (Westerman and Bicudo, 2005).

OW can be classified based on various characteristics, and different classifications can be useful for identifying the most appropriate end use or treatment method for the OW. For example, Ponsá et al. (2010) establish a qualitative classification of OW according to their biodegradability. In many circumstances, OW was classified on the basis of their sources (Dhaouadi, 2014): (i) agricultural sources, which are generated from farming and livestock operations, including livestock manure, slurries, and poultry litters; (2) municipal sources, which are generated from households and urban areas, including food waste, green waste, municipal solid waste, and sewage sludge; and (3) industrial sources, which are generated from manufacturing and processing industries, including food and paper processing waste and by-products.

1.2.2 Disposal approach

The OW disposal method must always focus on maximum human health and safety, minimum possible environmental impact, and effective reuse or recycling as much as possible (Ahring, 2003). According to these guidelines, various treatments method exist: (i) biological process, including composting or anaerobic digestion, and (ii) physical or chemical process, such as landfill, incineration, pyrolysis, liquefaction, and gasification. The typical treatment methods for OW are presented below.:

Anaerobic digestion

Anaerobic digestion is a complex microbial process under oxygen-free conditions, which has been used to stabilize sewage sludge for years and has now been expanded

to treat multiple OW, including animal manure, municipal solid waste, and organic industrial wastes (Ahring, 2003). During this process, OW is converted into an energy source (biogas) and a fertilizer (anaerobic digestate) (Nkoa, 2014).

The advantage of anaerobic digestion is that it produces energy and fertilizer simultaneously. Compared to incineration, anaerobic digestion is able to recycle the nutrients and produce more energy (Ahring, 2003); compared to composting, anaerobic digestion produces renewable energy and also reduces the odor and greenhouse gas (GHGs) emissions (Lin et al., 2018).

Composting

Composting is a natural aerobic microbiological process that converts organic matter into stable humic-like substances (Singh et al., 2010). Generally, raw materials used for composting include agricultural wastes (animal manure and urine), urban wastes (organic fraction of MSW, sewage sludge, and green waste), industry waste (wood processing residues, food processing residues, fermentation wastes, etc.), and local organic wastes (rice hulls, olive marc, etc.) (Senesi, 1989). Currently, several different systems are available for composting, including windrows, aerated static piles, bunkers, and in-vessel (enclosed) systems (Sánchez et al., 2017). To ensure an efficient composting process, optimal pH, porosity, water content, C/N ratio, temperature, and oxygen supply are required (Schaub and Leonard, 1996).

The advantage of composting is its low cost and technical difficulty. Compared to landfilling and incineration, considering the life cycle from the cradle to the grave, composting has the lowest use of resources and environmental cost (Marchettini et al., 2007; Valerio, 2010); compared to anaerobic digestion, composting can treat a broader range of raw materials, is more feasible to abate pathogens (Subirats et al., 2022), and is also advantageous in farmers' small-scale decentralized processing (Lin et al., 2019). Although CO₂ produced during composting is biogenic and not considered a greenhouse gas according to the IPCC (2006), the emissions of N₂O and CH₄ during the process significantly contribute to global warming. Some researches showed that during composting, approximately 0.2-9.9% of initial nitrogen is lost as N₂O (Chen et al., 2019), and 0.8-14% of initial carbon is emitted as CH₄ (Luo et al., 2013). Furthermore, since the composting process does not generate energy, it also results in a poor overall greenhouse gas balance when composting certain types of waste (Arafat et al., 2015).

Landfilling

The landfill is an area of land where waste is deposited and might be the simplest, lowest cost, and most common way for waste treatment (Singh et al., 2011). According to Singh et al. (2011), landfills can be classified into three categories: (i) Open dumps or open landfills; (ii) Semi-controlled or operated landfills; and (iii) Sanitary landfills. However, Europe has more than 500,000 landfills and 90% of them are old, non-sanitary landfills that lack the necessary technology for environmental protection

(EURELCO, 2018).

With the rise of sustainable waste management, the traditional landfill method is gradually being replaced by recycling methods such as composting, anaerobic digestion, and incineration. The disadvantages of landfilling, include space competition with settlements (Patil, 2014), pollution of the soil and groundwater by leachates (Gworek et al., 2016), impacts on air quality as well as human health (Dave et al., 2020), and absence of nutrient recycling.

Incineration

Incineration, also known as Waste-to-Energy, is a thermal treatment process where waste is burned in a specialized combustor to produce power and energy (Fodor and Klemeš, 2012). This approach is widely used in various waste treatments due to its high availability, reliability, waste flexibility, and energy efficiency (Di Maria et al., 2018). CEWEP (Confederation of European Waste-to-Energy Plants) reported that there were 492 waste-to-energy power plants in Europe in 2019, which supply enough electricity to 19 million people annually (CEWEP, 2019a, 2019b). Incineration has excellent efficiency in volume reduction. Incineration was reported to reduce the MSW volume by 95% (Abd Kadir et al., 2013).

Public concerns about incineration are mainly focused on health and environmental impacts. Incineration produce residues such as fly ashes and bottom ashes, which need further treatment (Fodor and Klemeš, 2012). Simultaneously, incinerators are a significant source of dioxins and other persistent organic pollutants (Makarichi et al., 2018). A range of adverse health outcomes such as congenital anomalies, miscarriage and some neoplasia has been shown to be associated with waste incineration (Tait et al., 2020). Furthermore, in the absence of nutrient recovery, incineration is considered one of the least desirable methods of disposal in agriculture.

Direct land application

The direct land application is the most traditional and cost-effective method, which can improve and sustain soil fertility. Compared with other treatment methods, direct land application have the lowest costs of shipping, storage, and processing.

However, the most significant concerns about OW direct land application come from their unknown composition in pathogens, toxic compounds, weed seeds, heavy metals, and foul odors (Ahmad et al., 2007). Untreated sludges showed the highest toxicity for all bioassays compared with digested, dried, and composted sludges (Roig et al., 2012). Although storing OW for an extended period of time before application can reduce the levels of some pathogens, it's not always a guarantee that it will completely prevent the spread of pathogens (Avery et al., 2005).

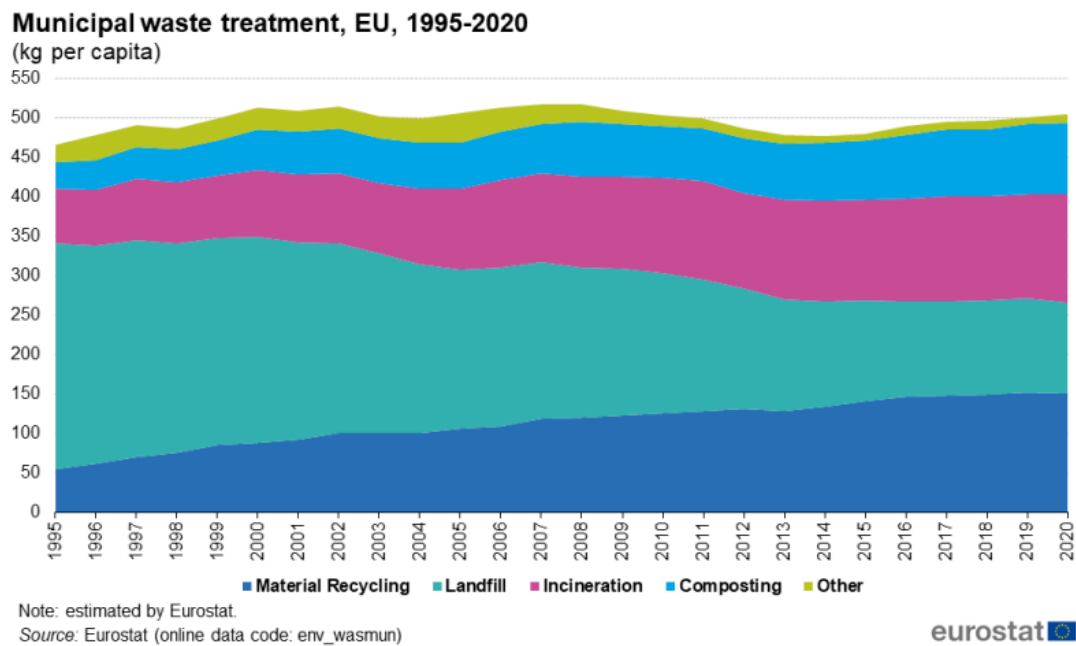


Figure 1-4 Municipal waste treatment, EU, 1995-2020 (Eurostat, 2021).

There are no detailed data on the treatment of OW due to its broad sources and the lack of a clear definition. Here we show how the disposal of municipal waste, which is an important part of OW, has evolved in the EU in recent years (Figure 1-4). The decline in landfill is particularly striking, with a significant drop from 286 kg per capita in 1995 to 115 kg per capita in 2020. Incineration, composting, and recycling have all increased steadily over this period. The change indicates a growing recognition of the need to recycle waste.

1.2.3 Principal regulations concerning OW recycling in agriculture

1.2.3.1 Regulations in European Union

Waste Framework Directive (75/442/EEC) is the first policy concerning waste management and was repealed as of 12 December 2010 by Directive 2006/12/EC. After that, numerous directives related to waste came into force, including the Landfill Directive (1999/31/EC) on the landfill of waste, the Animal Waste Directive (90/667/EEC) on disposal and processing of animal waste, Sewage Sludge Directive (86/278/EEC) on sewage sludge treatment and use and so on. The most important EU regulations in force about OW management and agriculture use are the following:

Waste Framework Directive (2008/98/EC)

Directive 2008/98/EC of the European Parliament and of the Council of 19 November 2008 establishes a framework for waste management in the EU. This directive repealed and replaced the previous Waste Oils Directive (75/439/EEC), Hazardous Waste Directive (91/689/EEC) and Waste Framework Directive (2006/12/EC). It aimed to "Protect the environment and human health by preventing or reducing the adverse

impacts of the generation and management of waste and by reducing overall impacts of resource use and improving the efficiency of such use." More specifically, the directive introduced the concept of "waste hierarchy" and "End-of-waste status":

Article 4, Waste hierarchy, establishes the following waste hierarchy in order of descending priority: prevention, preparing for re-use, recycling, other recovery, and disposal.

Article 6, End-of-waste status, specifies when certain waste undergone recovery and re-use would cease to be waste and therefore highlight the importance of waste recovery.

On 14 June 2018, Directive 2018/851 - Amendment of Directive 2008/98/EC on waste was published and entered into force on 4 July 2018. The new Amendment of Directive strengthens rules on waste prevention and sets new targets as at least 55% of municipal waste has to be recycled and 65% by 2035. This legislation could increase the amount of urban organic waste recycled in agriculture.

Sewage Sludge Directive (86/278/EEC)

On 12 June 1986, the Sewage Sludge Directive (86/278/EEC) entered into force. This Directive aimed to "*Regulate the use of sewage sludge in agriculture in such a way as to prevent harmful effects on soil, vegetation, animals and man thereby encouraging the correct use of such sewage sludge.*" The directive prescribes that sludge must be treated to reduce its fermentability and health hazards before agricultural use and restricts the maximum concentration of heavy metal (Cd, Cu, Hg, Ni, Pb, and Zn) in the soil, sludge, and annual sludge applied. It also highlights some particular situations in which sludge cannot be used in agriculture:

- Before a certain period of grassland that is going to be grazed by animals or the forage crops to be harvested has elapsed (at least 3 weeks);
- The growing season of fruit and vegetable (except the fruit trees);
- The fruit and vegetable crops that are in direct contact with soil and eaten raw (10 months before and during harvest).

Nitrates Directive (91/676/EEC)

The Nitrates Directive 91/676/EEC, implemented in 1991, is one of the critical parts of the Water Framework Directive (Directive 2000/60/EC) that aims to reduce nitrate contamination that occurs in water caused by agricultural sources and establishes preventive measures. According to the Directive, the following actions need to be applied in EU nations:

- Identification of the polluted water or risk
- Designation of the nitrate-vulnerable zones

- Regular monitoring of water bodies
- Establishment of codes of agricultural practices

This directive especially prescribes that the maximum amount of manure applied annually should not exceed 170 kg N ha⁻¹ in agricultural areas identified as polluted by nitrates.

Regulation (EU) 2019/1009

On 16 July 2022, Regulation (EU) 2019/1009 has replaced and repealed Regulation (EC) No 2003/2003. The new regulation lays down rules on safety, quality and labelling requirements for fertilizing products. The fertilizing products in this new regulation were defined as "*a substance, mixture, micro-organism or any other material, applied or intended to be applied on plants or their rhizosphere or on mushrooms or their mycosphere, or intended to constitute the rhizosphere or mycosphere, either on its own or mixed with another material, for the purpose of providing the plants or mushrooms with nutrient or improving their nutrition efficiency*". This regulation also expands the coverage of harmonization rules to include fertilizing products that were not previously subject to such rules. Based on the product function categories (PFCs), the new regulation covers seven categories of fertilizing products, including:

- Fertilizers (inorganic, organo-mineral, and organic);
- Soil improvers;
- Liming materials;
- Growing media;
- Inhibitors;
- Plant biostimulants;
- Fertilising product blends.

Moreover, the new regulation introduces limits for toxic contaminants and pathogens of fertilizing products.

1.2.3.2 Regulations in France

In France case, OW has different regulatory statuses depending on whether they are considered as waste, by-products or products. The regulatory frameworks of OW were governed by French Rural and Maritime Fishing Code (Code rural et de la pêche maritime), and French Environmental Code (Code de l'environnement). The goal of the law is twofold: to ensure that the fertilizers used are sufficiently effective and to prevent the risks that their use may pose to human health and the environment.

Waste

According to the Article L. 541-1-1 of the Environmental Code, waste is defined as "any substance or object that the holder discards or intends or is required to discard". The list of wastes is given in Appendix II of Article R. 541-8 of the Environmental Code.

However, the list is not exhaustive and the definition of OW is not defined in the code.

In particular, sludge regulations (order of January 8, 1998) set the technical requirements applicable to the spreading of sludge on agricultural soils, which including the thresholds of 7 heavy metals (Cd, Cr, Co, Hg, Ni, Pb, and Zn), 3 polycyclic aromatic hydrocarbons (PAHs) and 7 polychlorinated biphenyls (PCBs).

By-products

According to the Article L. 541-4-2 of the Environmental Code, a substance or object resulting from a production process whose primary purpose is not the production of that substance or object may only be considered as a by-product when they meet the following conditions:

- Produced as an integral part of a production process;
- Can be used directly without further processing (except normal industrial practices);
- The subsequent use is certain and meets all product, environmental, and health protection requirements.

In particular, straw and various agricultural effluents are considered by-products when they are used in the context of a farm, whether or not it has produced them.

Products

The standard (NF U 42-001) includes organic fertilizers entirely of animal and/or vegetable origin and organo-mineral fertilizers. To qualify for this standard, the product must correspond to one of the denominations while respecting the minimum thresholds set for a few agronomic parameters. Apart from that, the standard (NF U 44-095) mainly concerns composts made from municipal sludge and certain industrial sludge while the standard (NF U 44-051) mainly covers products made from plant and animal waste and urban compost made from household waste. These two standards specify the dry matter content of OW shall be higher than 30% of raw material. In addition, both standards set limits for metallic trace elements, organic trace compounds, microorganisms, and impurities, but the specific limits differ between the two standards (Table 1-1). If this requirement is met, the OW can be considered as a "product" status otherwise it remains a "waste" status.

Table 1-1 Brief comparison of the standard NF U 44-051 and the standard NF U 44-095.

	NF U 44-095	NF U 44-051
Agronomic thresholds	Dry matter content Organic matter content OM / Organic N < 40 N, P ₂ O ₅ , K ₂ O content	Dry matter content Organic matter content C / N ratio > 8 N, P ₂ O ₅ , K ₂ O content
Metallic trace elements	Maximum content and flux of As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn	Maximum content and flux of As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn
Trace organic compounds	Polychlorinated biphenyls (PCBs) Polycyclic aromatic hydrocarbons (PAHs)	Polycyclic aromatic hydrocarbons (PAHs)
Micro-organisms	Escherichia coli Clostridium perfringens Enterococci Listeria monocytogenes Viable helminth eggs Salmonella	Viable helminth eggs Salmonella
Inert matter and impurities		Plastic film, expanded polystyrene, other plastics, glass and metal

In addition, the French Ministry of Agriculture and Food (MAA) and Ministry of Ecological Transition (MTE) have drafted a decree called "socle commun" outlining agronomic quality and safety criteria for fertilizing materials and growth media (Matières fertilisantes et supports de culture – MFSC). The draft has been circulated to stakeholders for comments, and aims to create categories of fertilising materials and provide rules concerning their effectiveness, safety, traceability, and use. The aim of "socle commun" is to regulate soil and crop contamination through fertilization practices by establishing consistent quality standards for all fertilizing materials, taking into account their specific characteristics. This will involve harmonizing regulations for all materials, adapting traceability systems to ensure quality control, and providing information to users such as farmers and food producers to assist them in making informed decisions and reducing soil and crop contamination. The application of fertilizing materials should be adjusted according to their quality, and the safety criteria should be updated regularly based on scientific advancements.

Regulation of application in areas vulnerable to nitrate pollution

Articles R. 211-80 and Articles R. 211-81 of the Environmental Code prescribed the requirements of N fertilizer spreading in areas vulnerable to nitrate pollution, in application of the European nitrate directive. Three kinds of N fertilizers including Type I (high C/N fertilizers), Type II (low C/N fertilizers) and Type III (synthetic mineral and urea nitrogen fertilizers) are distinguished according to these articles. Fertilizer ban periods were also established depending on the landuse, crop type, and the fertilizer type (Table 1-2). Moreover, it limits N fertilizer application according to the balance between the crop's N demand and various N inputs. The amount of N applied to

agricultural land by OW is limited to 170 kg N per hectare.

Table 1-2 Nitrogen fertilizers ban period in vulnerable areas (Directive of 23 October 2013, article 3 and Directive of 11 October 2016, article 2).

Land use (main crop)	Types of Nitrogen fertilizers			
	Type I		Type II	Type III
	Manure not liable to flow and composts of manure	Other Type I Effluents		
Uncultivated	All year		All year	All year
Crops in autumn or late summer (except rapeseed)	15/11 to 15/01		01/10 to 15/01	01/09 to 31/01
Rapeseed in the autumn	15/11 to 15/01		15/10 to 31/01	01/09 to 31/01
Crops planted in the spring not preceded by cover crops	01/07 to 31/08 and 15/11 to 15/01	01/07 to 15/01	01/07 to 31/01	01/07 to 15/02
Crops in the spring preceded by cover crops	20 days before cover crops harvest to 15/01	01/07 to 15 days before cover crops planted and 20 days before cover crops harvest to 15/01	01/07 to 15 days before cover crops planted and 20 days before cover crops harvest to 31/01	01/07 to 15/02
The total of the N inputs before and on the cover crops is limited to 70 kg of available N/ha				
Grassland more than 6 months (grassland, alfalfa)	15/12 to 15/01		15/11 to 15/01	01/10 to 31/01
Other crops	15/12 to 15/01			

1.2.4 Benefits of OW recycling in agriculture

Recycling various sources of OW as fertilizers in agricultural production is a long-established farming practice worldwide. However, from the 1870s onward, recycling OW in agriculture were gradually replaced by the rapid development of industry and the fertilizer revolution (Barles, 2014). In recent years, environmental and energy problems caused by chemical fertilizers are becoming more prominent. OW agricultural use has gradually come back into the limelight due to its great potential for increasing soil fertility and closing nutrient cycles. Over 50% of produced sewage sludge (European commission, 2021) and 90% of animal manure (Königer et al., 2021) are recycled into agriculture as organic fertilizer in the European Union. In a recent report, the French Environment and Energy Management Agency (ADEME) indicated that 729 Mt of OW are generated annually, of which roughly 300 Mt is utilized in agriculture as organic amendments and fertilizers, with 94% of them being livestock effluents. About 73% of generated sewage sludge (31% after composting), 35% of organic industrial waste (8% after composting), and 14.5% of household waste

composted have been applied on a total of 6.6 Mha of field crops and grasslands (ADEME 2018).

Recycling OW in agriculture has demonstrated two different potential uses: (I) as fertilizer, which recycles valuable nutrients (mainly N, P, and K) thereby substituting mineral fertilizers; and (II) as a soil amendment, enhancing soil organic carbon (SOC) and improving chemical, physical and biological soil properties. For the different OW, the fertilizer and amendment values varied greatly, depending on the type of OW (Figure 1-5) (Houot et al., 2014).

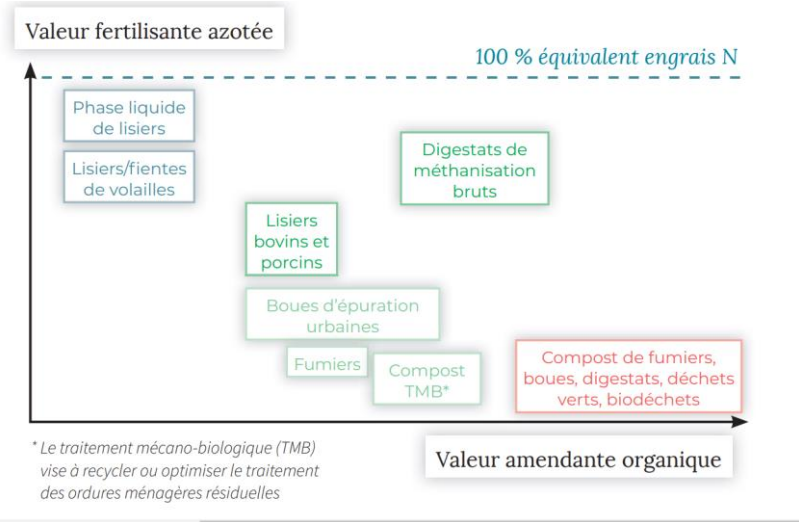


Figure 1-5 Fertilizer and amendment value for several organic wastes (Houot et al., 2014).

1.2.4.1 OW as fertilizer

One of the most significant interests in recycling OW in the soil comes from its ability to supplement the soil supply of available nutrients for crops, especially for the N, P, and K. OW application significantly increased soil N content which is essential for plant growth. However, a considerable portion of the addition of N occurs in forms not readily available to plants and needs to be converted into plant-available inorganic forms by mineralization (Rayne and Aula, 2020). The availability and release rate of nitrogen (N) in different types of OW vary significantly (Levavasseur et al., 2022). For example, only about 20% of the total N in compost (Eghball et al., 2004), 25% in fresh manure (Amlinger et al., 2003), and 55% in sludge (Magdoff and Amadon, 1980a) is available to plants in the first year. To evaluate the effect of different types of OW on crop yields under various conditions, "Fertilizer replacement values" (FRV) or "Mineral-fertilizer equivalents" (MFE) are often used. These values are calculated relatively to the performance of mineral fertilizers and can vary greatly. Gutser et al. (2005) summarized the MFE for several organic fertilizers (Figure 1-6).

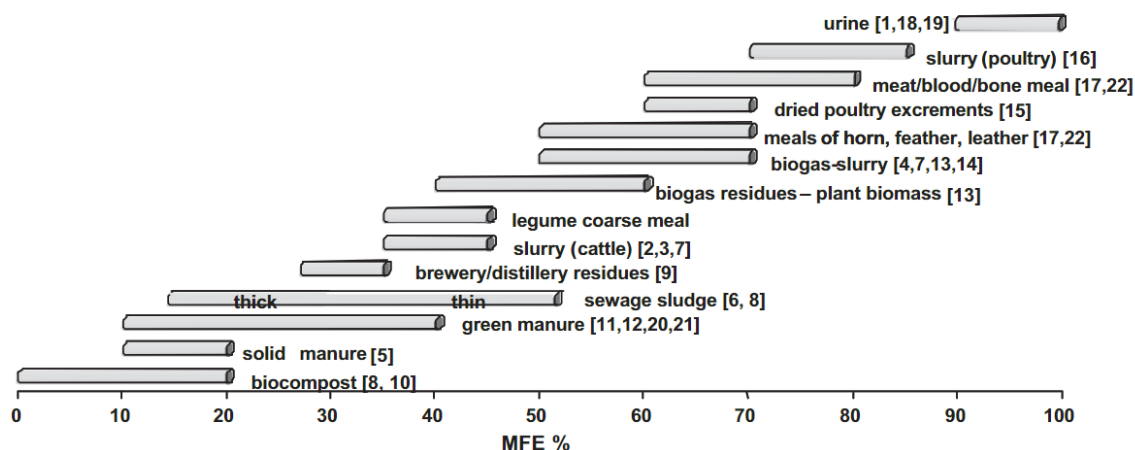


Figure 1-6 Mineral-fertilizer equivalents (%MFE) for several organic fertilizers characterizing N availability in the year of application (Gutser et al., 2005).

OW also contains a large amount of phosphorus (P) and potassium (K) that can be used to replace mineral fertilizers. It was reported that P and K requirements for crops would be satisfied or even excess on a long-term basis when N requirements are supplied by OW (Olsen and Barber, 1977). Even with an equivalent P input, OW application can result in a higher proportion of available P compared with mineral P fertilizer (Yan et al., 2017). OW influence the available P concentration by at least two different mechanisms: by directly introducing organic and inorganic P compounds and indirectly by affecting the P availability in the soil with the alteration of the dissolution and desorption of P minerals (Wang et al., 2022). Furthermore, OW application can result in a higher amount of available K in the soil compared to the use of mineral fertilizers (Herencia et al., 2007). Blake et al. (1999) carried out a study based on three European long-term field experiments and reported that in addition to being a source of K, FYM also increased soil CEC, leading to an increase in soil K availability and plant use efficiency.

1.2.4.2 OW as a soil amendment

1.2.4.2.1 OW effects on soil organic matter

Soil organic matter (SOM) plays a crucial role in terrestrial ecosystem processes and is sensitive to management. It acts as a reservoir for nutrients, improves soil structure and water-holding capacity, and enhances soil biodiversity (Abdallah et al., 2021). In addition, sequestration of atmospheric C in SOM by OW application can offset GHGs emissions and reduce global warming (Lal, 2009).

Regular application of OW promotes the accumulation of soil organic matter (SOM) and improves SOM quality, which highly depends on the rate of application (Delschen, 1999). The accumulation of soil organic matter also depends on the type of OW (Levavasseur et al., 2020). Different types of OW also lead to differences in the

composition of SOM in the long term. Monaco et al. (2008) found that the use of FYM caused a more significant SOM increase compared to the slurry. Peltre et al. (2017) reported that compost and cattle manure amended soils showed greater SOM thermal stability compared with sludge while compost had greater biological stability than cattle manure in amended soils.

1.2.4.2.2 OW effects on soil physicochemical properties

Repeated OW application has been reported to positively affect soil physicochemical properties in both short-term and long-term studies (Paradelo et al., 2019; Shepherd et al., 2002). OW application can increase SOC, which is a keystone factor for soil structure evolution (Zhou et al., 2020). Soil bulk densities are usually negatively correlated with the SOC content (Shepherd et al., 2002) and decrease with OW application can also be due to the dilution effect of the less dense organic material mixing with the denser mineral fraction of the soil (Khaleel et al., 1981). Soil water holding capacity is primarily controlled by the soil structure, especially the specific surface area, pore size, pore numbers, and density (Khaleel et al., 1981). Repeated applications of OW can thus slightly increase soil water holding capacity (Eden et al., 2017). Soil aggregate stability, generally strongly correlated with SOC, is a crucial soil property affecting soil sustainability and crop production (Amézqueta, 1999). The organic matter stabilizes soil structure by enhancing cohesion within aggregates and decreasing the wettability of aggregates (Diacono and Montemurro, 2010). Annabi et al. (2011) showed an increase in soil aggregate stability with repeated OW applications. In addition, an increase in soil organic matter also increases pH buffering capacity (Shi et al., 2019) and thereby increases the pH of acid soils (Whalen et al., 2000) or counteracting the pH drop caused by inorganic nitrogen fertilizers (Magdoff and Amadon, 1980b). Soil cation exchange capacity (CEC) is closely related to soil fertility and tends to increase with an increase in SOC content (Gao and Chang, 1996). Previous studies have well demonstrated the positive effects of OW application on soil CEC (Epstein et al., 1976; Perez-Espinosa et al., 2000). However, the increase in soil CEC may not be immediately apparent, and it may need several applications of OW over time before it becomes noticeable (Whalen et al., 2000). This has also been demonstrated by another study carried out by Miller et al. (2016), who found that soil CEC was unchanged after one year of OW application, but it significantly increased after eight years, with the differences becoming greater as time passed. Furthermore, an increase in SOM can lead to an increase in soil organic N mineralization, providing a long-term source of nitrogen for crops (Chalhoub et al., 2013).

1.2.4.2.3 OW effects on soil biology

Soil microorganisms

Soil microorganisms drive many vital functions in the soil, such as nutrient cycling and the degradation of organic matter (de Araújo et al., 2010). Many parameters related to soil microorganisms could be used as sensitive indicators of soil quality, including the

biomass and diversity of specific functional microbial groups (Bending et al., 2004; Turco et al., 1994). Among them, bacteria and fungi are the most common microbial groups in the soil, and it is estimated that millions of them live in a single gram of soil (Sabir et al., 2021). Analyzing the activity, biomass, and community structure of soil bacteria and fungi provides insight into the response of the soil to different management practices (Schloter et al., 2003).

Extensive research has found that OW application favors soil microbial diversity and activity (Morugán-Coronado et al., 2022; Sabir et al., 2021; Sadet-Bourgeteau et al., 2019). The increase in soil microbial biomass is mainly due to the exogenous organic C additions that stimulate the indigenous soil microbes and also the microbial biomass contained in the OW (García-Gil et al., 2000). It is generally accepted that soil microbial biomass increases with the application of OW and is positively correlated with C input (Houot and Chaussod, 1995; Kallenbach and Grandy, 2011). However, results on how OW affect bacteria and fungi are not always consistent. Zhang et al. (2012) reported that organic manures increased bacterial and fungal populations and their diversity. A meta-analysis carried out by Bebber and Richards (2022) showed that organic input increased bacterial diversity by 3.8% and 7.0% compared to mineral fertilizer and no fertilizer, respectively, whereas fungal diversity did not differ between all the treatments. Zhang et al. (2015) found that OW significantly lowered the abundance of fungi and suggested that this was due to the competition between bacteria and fungi for substances. Kirchmann et al. (2004) reported that after 42 years OW application, neither the quality nor the quantity of organic matter input did not affect soil bacteria community structure.

Soil enzymes

Soil enzymes are essential for soil biochemical functions and nutrient cycling because all biochemical transformations (e.g., mineralization and decomposition) in soil depend on or are related to the presence of enzymes (Pascual et al., 1998). Thus, soil enzyme activities have been suggested as important indicators of soil quality and health because of their immediate response to soil change and ease of measurement (Bandyopadhyay and Maiti, 2021). For example, dehydrogenase is commonly used as an indicator of overall microbial activity; β -Glucosidase is widely used for evaluating soil quality and the C cycle; urease, as well as arylamidase activity, reflects the situation of N cycles; Arylsulfatase, which catalyzes the hydrolysis of sulfate esters, is a key component in the S cycle; phosphatases, including acid phosphatase and alkaline phosphatase, are believed to play critical roles in P cycles (Adetunji et al., 2017; Riah et al., 2014).

OW application contributed to the increase in soil enzyme activity (Utobo and Tewari, 2015). Chang et al. (2007) reported that the dehydrogenase, urease, β -glucosidase, and arylsulfatase activities were significantly linearly correlated with soil organic matter contents. In addition, soil pH changes soil enzyme activities by altering the ionization

and solubility of the soil enzyme substrates and enzyme factors (Tabatabai, 1994). Besides, the intra- and extracellular enzymes within OW may also stimulate enzymatic activities in the soil (Pascual et al., 1998). Several studies demonstrated that OW input increases the concentration and availability of trace metals which have side effects on enzymatic activity (Frankenberger et al., 1983; Moreno et al., 2003). Trace metals can cause inhibition of enzyme activities by directly reacting with soil enzymes and indirectly affecting soil microbes that are related to enzyme activities (Kızılkaya and Bayraklı, 2005). However, these adverse effects of trace metals strongly depend on their amounts and could be masked by the positive effect of OW application (Albiach et al., 2000; Kızılkaya and Bayraklı, 2005).

Soil Nematodes

Nematodes are one of the largest phyla on Earth and occupy a central position in the soil food web (Lazarova et al., 2021). Thus, studying the composition and functions of the nematode community can provide insight into soil ecosystem structure and soil health (Biederman et al., 2008). Nematodes can be assigned to the following trophic groups according to their feeding habits (Yeates et al., 1993): bacterivores (BF), fungivores (FF), omnivores-predators (OP), and plant-parasites (PP). Among them, plant-parasitic nematodes pose a severe threat to agricultural production which caused 8.8–14.6% of annual yield loss globally (Singh et al., 2013). Numerous nematodes community indices can be used to assess and monitor soil condition, including enrichment index (EI), which indicate soil nutrient; structure index (SI), which reflect soil food web complexity; maturity index (MI) and plant parasite index (PPI), which indicate the extent of soil community disturbance; and nematode channel ratio (NCR), which is related to decomposition pathways in the soil (Biswal, 2022).

OW application can affect the nematode community through supplying nutrients and changing soil properties (Oka, 2010). Results of the meta-analysis carried out by Puissant et al. (2021) showed that OW application enhanced all trophic groups of nematodes as well as diversity. Pan et al. (2020) found that OW affected nematode community structure and metabolic footprint, both bacterivores and fungivores increased with OW application while plant parasites decreased. On the contrary, Zhang et al. (2019) reported that organic input leads to an increase in bacterivores and decreasing in fungivores. In summary, OW application appears to have a significant and lasting effect on nematodes, although it varies between the type of OW, soil, and plant (Li et al., 2018; Puissant et al., 2021).

1.2.4.3 OW effects on crop yields

As a result of the supply of nutrients and the improvement of soil properties, recycling OW significantly increases crop yields compared to no fertilizer application. However, in comparison to conventional synthetic fertilizers, OW application might decrease yields (Diacono and Montemurro, 2010; Edmeades, 2003; Zhang et al., 2020). For example, Zavattaro et al. (2017) reviewed 80 long-term European field trials and found

that when applied at similar rates of N application, the application of farmyard manure and slurry alone could reduce yields by approximately 9% compared to mineral fertilizers. The exact effect of OW on crop yields is highly dependent on factors such as crop types (Chen et al., 2018), OW types (Geng et al., 2019), soil and climates conditions (Du et al., 2020) as well as management practices (Wang et al., 2020). Although there is a risk of reduced yields compared to conventional fertilizers, the lasting beneficial effects of OW on crop productivity cannot be ignored. Due to the slow release of nutrients as well as the improvement of soil properties, organic fertilizers continue to have a positive impact on crop yields even after 1-16 years of discontinuation (Eghball et al., 2004; Obour et al., 2017).

Organic fertilizers generally have low levels of plant-available nitrogen and low spring temperatures restrict nitrogen mineralization, limiting the yield of organic crops (Röös et al., 2018). In order to mitigate this limitation and achieve greater yield output, partial substitution of mineral fertilizers by OW is considered to be a more practical approach (Zhang et al., 2020). However, the results of studies on the effect of partial substitution have been inconsistent, with some finding that yield increases (Li et al., 2022), remains stable (Zhou et al., 2016), or decreases (Fan et al., 2017) compared with mineral N treatment. In addition to the factors such as crop type and soil and climatic conditions, the effect of partial substitution highly depends on the substitution rate and the type of OW (Geng et al., 2019). For example, Tong et al. (2022) reported that partial substitution of synthetic fertilizers with OW at the rate of 25% significantly increased the yield of wheat and maize, while substitution rates of 50% or higher resulted in a yield decrease. Similarly, Xia et al. (2017) analyzed 141 relevant studies and summarized that the optimum rate for manure substitution for fertilizer is 50-75%, while substitution rates above 75% can lead to reduced yields.

1.2.5 Environmental impacts

1.2.5.1 Trace elements

Trace elements are defined as microelements at a low concentration that can be contained in or adsorbed to most soil minerals (He et al., 2005). The trace elements (TE) including Cu, Zn, Fe, Mn, Mo, and B play an important role in the growth and productivity of plants and other living organisms, while some of them such as Cu, Mo, and Zn can be harmful in large quantities (He et al., 2005). Additionally, elements such as Cd, Cr, Pb, As, Hg, Ni, and Se have been identified as hazardous to human health (Okerefor et al., 2020). There have been some investigations remarking heavy metal accumulation problems in OW applied soils, such as animal manure (Zhao et al., 2014), sewage sludge (Wang et al., 2008), composted (Michaud et al., 2020) and municipal solid waste composts (Yuksel, 2015). However, the trace element content in the soil is not always easily detectable with a single application but may accumulate to harmful levels in the cropland through repeated applications (Brock et al., 2006). Potentially available TE increase gradually in the topsoil, the growth rate depending on the

element and type of OW (Cambier et al., 2019). In France, it was reported that the primary source of most TE in arable land is animal effluent (Figure 1-7) (Belon et al., 2012), which is mainly because animal feeds are often supplemented with TE to maintain various physiological processes and prevent health disorders (Benke et al., 2008). Not only that, but elevated levels of heavy metals in the soil can also lead to accumulation in plants. It was reported that sewage sludge amendment led to an increased concentration of TE in soil and resulted in higher levels of Cr, Cu, Ni, and Zn in sunflower shoots and roots (belhaj et al., 2016). Similar results were also reported by Singh and Agrawal (2007), who found sewage sludge amendment resulted in the concentrations of Cd, Ni, and Zn in leafy vegetable palak exceeding even the permissible limits of Indian standards. This can lead to environmental pollution and potential harm to human health throughout the food chain. Heavy metal accumulation in the body can negatively impact the central nervous system and contribute to the development of various health issues (Alengebawy et al., 2021).

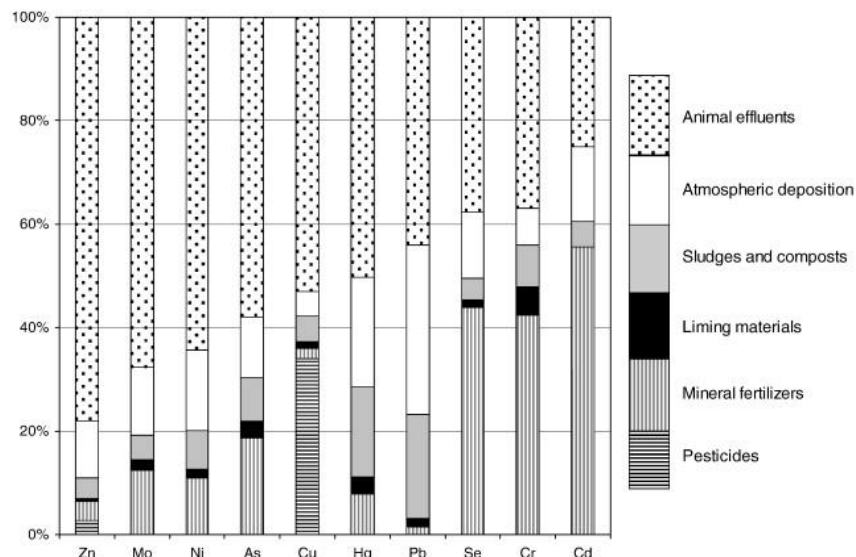


Figure 1-7 Annual contribution of six contamination sources to the average total amount of 10 TEs reaching French soils. (Belon et al., 2012).

1.2.5.2 Pathogens and Pharmaceuticals

To improve production performance in modern animal husbandry, more than 70% of medical antibiotics are used in livestock husbandry (Li et al., 2017). However, large proportions (17%–90%) of undegraded antibiotics are excreted directly through urine or feces and reach agricultural fields by manure fertilization (Massé et al., 2014). Apart from animal excreta, unused and incompletely metabolized antibiotics are also discharged into the sewage system and are found in sewage sludge (Kumar and Pal, 2018). Some synthetic antibiotics are highly resistant to biodegradation and can persist for months to years in soil (Du and Liu, 2012). Moreover, repeated application of OW tends to enhance antibiotic sorption and reduce degradation by increasing the quantity and quality of SOM (Andriamalala et al., 2018). Once antibiotics enter the agricultural land, crops exposed to antibiotics may become contaminated, potentially putting

human health at risk through prolonged exposure (Boxall et al., 2006). These risks include chronic toxic effects, allergic reactions, development of antibiotic-resistant (AR) bacteria, and disorganization of digestive system (Dolliver et al., 2007). In addition to antibiotics, considerable numbers of antibiotic resistance genes (ARGs), associated mobile genetic elements (MGEs), and other kinds of pharmaceuticals are introduced to the soil via OW application (Boxall et al., 2006; Jechalke et al., 2014).

Pathogens such as bacteria, viruses, and parasites are another primary concern regarding OW application. Approximately 70% of foodborne pathogens are sustained in animals and can be transferred to soil by manure or slurry land application (Behravesh et al., 2012). The pathogens in the soil can cause tetanus and hookworm infections, which can enter the body through abrasions and cause other diseases (Oliver, 1997). Several previous experiments have also revealed that pathogens can be transferred from contaminated soil to plants (Mootian et al., 2009; Solomon et al., 2002). For example, Looper et al. (2009) applied contaminated manure slurry to soil and found positive *Escherichia coli* O157:H7 in fescue plants.

OW treatments such as anaerobic digestion (Seruga et al., 2020) and composting (Dumontet et al., 1999) can effectively reduce pathogens counts. However, it is worth noting that the handling of OW can sometimes be a double-edged sword. Improper handling during the composting process can lead to secondary contamination, which can be a source of dangerous pathogens (Dumontet et al., 1999).

1.2.5.3 Greenhouse gas emission

Three common anthropogenic gases, carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O), are the primary greenhouse gases (GHGs) associated with agriculture. Among them, soil CH₄ release was negligible in terms of total greenhouse gas emission, particularly in the case of upland crops (Ball et al., 2004; Flessa et al., 2002). OW application usually leads to an increase in soil CO₂ emission (Cayuela et al., 2010; Ding et al., 2007; Shakoor et al., 2021). Rochette et al. (2006) reported that both liquid and solid manures led to more significant CO₂ emission than mineral fertilizer when applied at an equivalent N rate (150 kg/ha). The increase in CO₂ emission is mainly due to the extra decomposition of exogenous organic C, which was introduced by OW (Thangarajan et al., 2013). Furthermore, OW application can induce a positive priming effect of native soil organic matter, which leads to increased soil carbon mineralization and subsequently accelerates the decomposition of soil organic matter (Wu et al., 2018).

There are no universally accepted conclusions about whether OW leads to higher N₂O emissions in comparison with inorganic fertilizers. A global meta-analysis carried out by Zhou et al. (2017) showed that manure application increased soil N₂O emissions by 32.7% compared with synthetic N fertilizer, consistent with numerous field studies (Pelster et al., 2012; Senbayram et al., 2009). However, the opposite results were also reported in many case studies (Chantigny et al., 2010; López-Fernández et al., 2007; Velthof et al., 1996). For example, López-Fernández et al. (2007) found urea application

increased N₂O emissions compared with multiple types of OW (e.g., municipal solid waste, sheep manure, and pig slurry). Soil N₂O emissions resulted from nitrification and denitrification by microbial processes, which highly depend on the soil properties, cropping systems, climate conditions, and OW characteristics (Lazcano et al., 2021; Snyder et al., 2009; Zhou et al., 2017). Relative to synthetic N fertilizer alone, OW application increased the availability of labile organic C in soil (Senbayram et al., 2009). These labile organic C compounds serve as an energy source for microorganisms to increase the activity of heterotrophic denitrifying microorganisms and stimulate N₂O production (Köster et al., 2015; Lazcano et al., 2021). Furthermore, OW has the potential to decrease soil redox potentials thereby increasing the activity of denitrifiers and leading to N₂O emissions (Guenet et al., 2021). On the other hand, OW enhances microbial activity and creates anoxic conditions in the soil, thus triggering N₂O emission via facilitating denitrification (Pelster et al., 2012).

1.3 LONG TERM FIELD EXPERIMENTS (LTES) FOR STUDYING OW

1.3.1 Interest of LTES

Long-term field experiments (LTES) are an indispensable resource when assessing the long-term sustainability and productivity of agriculture systems (Bergkvist and Oborn, 2011). However, there is no clear definition of LTES, especially their specific duration. Knapp et al. (2012) defined LTE as an experiment planned to exceed or already exceed six years. BonaRes (Soil as a Sustainable Resource for the Bioeconomy) has defined LTE as a trial that has a static design and focuses on a scientific issue in the context of soil and yield with a minimum duration of 20 years (Grosse et al., 2020). Regardless of the specific duration, treatments within LTES are generally kept constant depending on the research targets. Thus, the cumulative effects and processes taking several years to become evident can be studied (Reckling et al., 2021).

Modern LTES date back to the mid-1800s. Lawes and Gilbert created Rothamsted Broadbalk continuous wheat LTES in 1843, considered as the beginning of modern LTE research. Since then, more and more LTES for different purposes have been initiated worldwide, primarily used to assess the impact of different management practices (e.g., fertilization, tillage, crop rotation) on soils, plants, and the environment. More extensive interest has also been involved in LTES, such as species invasions (Burke et al., 2019), pest management (Lechenet et al., 2017), and climatic changes (Bradford et al., 2008). According to previous statistics, there have been more than 600 LTES over 20 years in the world nowadays, and this number is overgrowing (Körschens, 2006). A recent study shows that there are more than 600 LTES in Europe and a considerable number of them are investigating the impact of fertilization (Figure 1-8), including those that have already lasted for 20 years and those that are planned to do so (Donmez et al., 2022).

Compared with short-term experiments, the advantage of LTES is that they allow

researchers to observe the magnitude of long-term changes and to identify the causes of inflection points in those changes (Knapp et al., 2012). Comprehensive analysis of the results from multiple LTEs (e.g. meta-analysis) can help us to identify better patterns and trends that may not be evident in individual studies and understand the factors that influence the results of these experiments. In addition, LTEs provide a wealth of valuable data that can be used to develop and calibrate a range of models such as SOC dynamic (Levavasseur et al., 2020), GHGs emission (Ludwig et al., 2011), and crop yield (Zelenák et al., 2022).

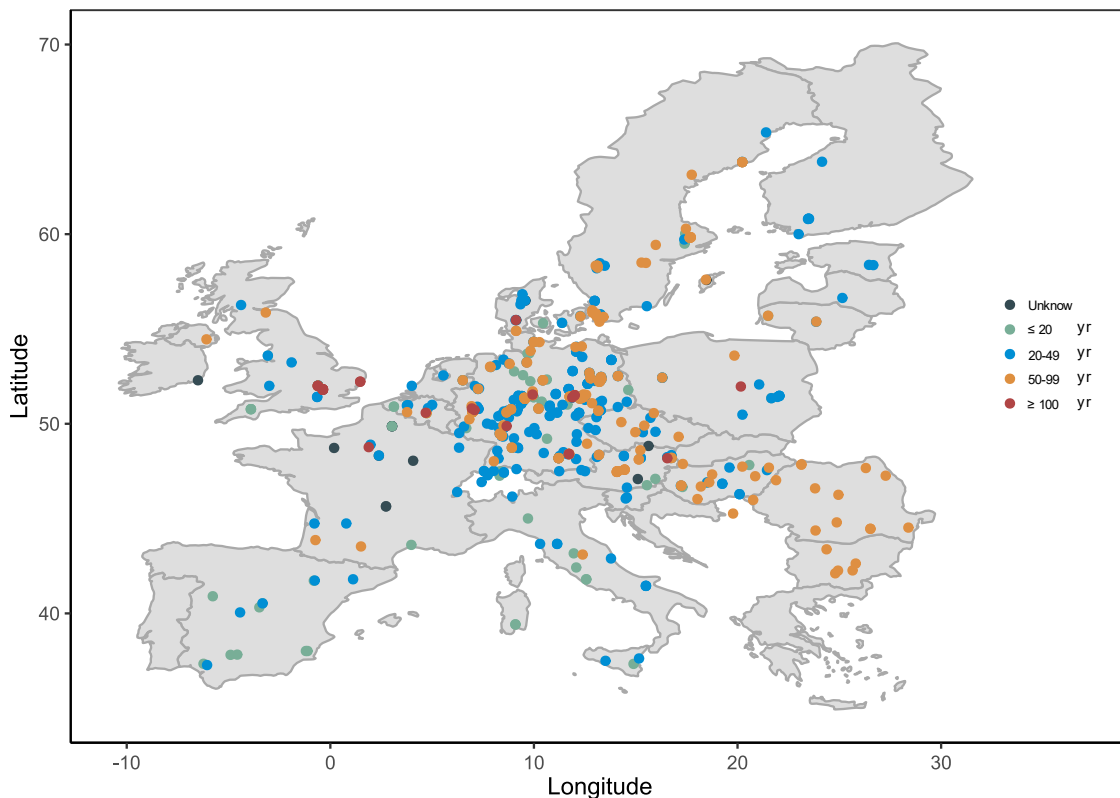


Figure 1-8 Long-term field experiments (LTEs) in EU-28. Data source: Donmez et al. (2022).

1.3.2 Example of the Rothamsted LTEs

The Rothamsted LTEs are located at Rothamsted, Hertfordshire, England, approximately 50 km north of central London. These experiments are some of the oldest and most well-known continuous studies of crop production in the world. In June 1843, John Bennet Lawes and Joseph Henry Gilbert started the first large-scale experiment on turnip on Barnfield, and in autumn the second on winter wheat on Broadbalk. Their initial interest was in studying crop response to mineral and organic fertilizers. Between 1843 and 1856, several experiments were established which are collectively known as the "Classical Experiments" (Macdonald et al., 2020): Barnfield (started in 1843), Broadbalk winter wheat (started in 1843), Hoosfield (started in 1852), Garden Clover (started in 1854), Park Grass (started in 1856), Alternate (start in 1856), and Exhaustion Land (start in 1856).

The earliest essential result from Rothamsted LTEs is that N fertilizers are necessary for good crop yields, but adding additional plant-available phosphorus can further increase yields (Johnston and Poulton, 2019). Another significant result obtained in the early stage is that FYM application at 35 t ha⁻¹ produced the same crop yield as mineral fertilizers (Figure 1-9). Subsequently, more extensive research findings based on Rothamsted experiments were reported, producing valuable information for farmers, agronomists, and decision-makers on management practice (Macdonald et al., 2020). For example, the LTE in Hoosfield and Broadbalk showed that SOC will approach equilibrium values after about 80–100 years of FYM annual application (Poulton et al., 2018). Extensive data about SOC from the Rothamsted LTEs have been used to develop the Rothamsted carbon model (Roth-C) and successfully simulated soil C dynamics at Rothamsted LTEs and elsewhere (MacDonald and Poulton, 2009). To date, there have been more than 1900 references to the Rothamsted LTEs and the meteorological records in articles, reports, or documents (e-RA; Rothamsted Research, 2023).

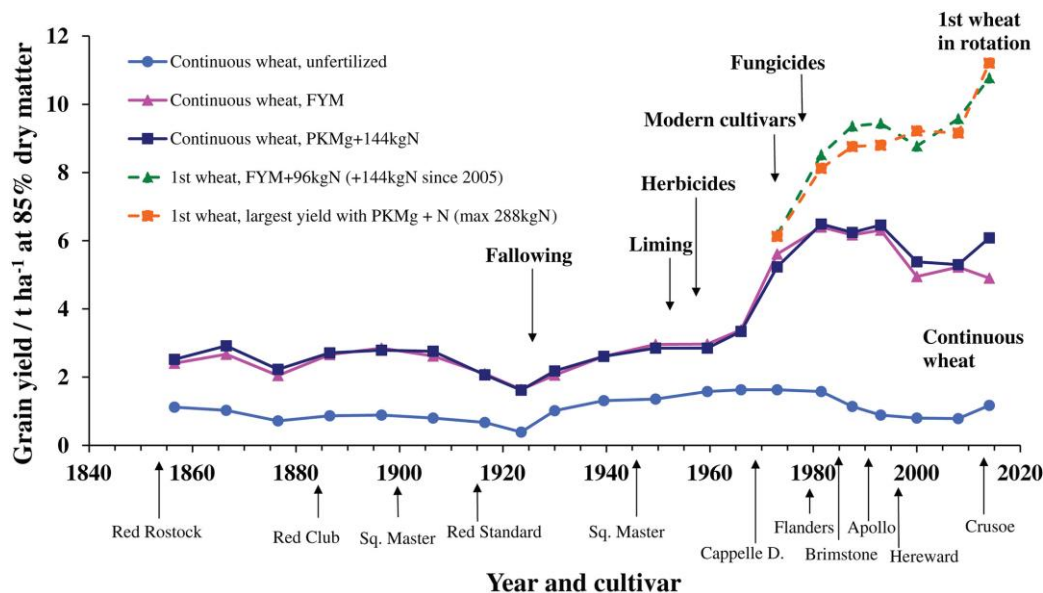


Figure 1-9 Mean long-term yields of winter wheat grain, 1852–2016, showing selected treatments, important changes in management, and cultivars grown. Broadbalk Winter Wheat experiment, Rothamsted. (Johnston and Poulton, 2018).

1.3.3 Limitations of LTEs

The biggest limitation of LTEs is that they are expensive and time-consuming to set up and maintain. In addition, some LTEs were designed without repetition or randomization, which can lead to biased estimates of treatment effects due to inherent differences between plots (McRae and Ryan, 1996). Despite the fact that up to 64% of peer-reviewed papers about LTEs are focused on fertilization (Berti et al., 2016), few LTEs compare various OW and use OW at actual farm practice rates. For example, the Rothamsted Broadbalk winter wheat experiment described in the previous section only has one level of the factor organic fertilization at 35 t FYM/ha per year. Another famous fertilization LTE Ultuna (located in Sweden) also applied large quantity OW at 4 t C/ha every second year and removed crop residues (Karhu et al., 2012). The management

strategies used in these experiments aim to isolate and accentuate the effects of OW, but they may not accurately reflect the methods used by farmers in real-world settings. The results of LTEs are usually applicable in a specific location and soil type, thus it may not be possible to generalize the results to a large scale (Hlisnikovský et al., 2022). Soils of the most well-known LTEs Rothamsted (initiated in 1894), Askov (initiated in 1894), and Ultuna (initiated in 1956) were classified as luvisol or cambisol according to the WRB-FAO classification system (IUSS Working Group WRB, 2015). Research on other soils, particularly calcareous soils, has been limited to our knowledge. Moreover, many works conducted on LTEs focus on specific topics (e.g., SOM, yield, aggregate stability) rather than analyzing multiple effects together. For this reason, it is difficult to identify what are the main effects and the interaction between these effects. As mentioned in previous sections, the effects after OW application highly depends on the type of OW. However, few LTEs have compared multiple OW at the same time, making it difficult to identify the drivers of these effects.

In addition, the analysis of LTEs data can be complex and challenging due to the potential for confounding factors such as changes in treatments and agronomic management (Reckling et al., 2021). Data collected from LTEs may not necessarily follow a normal distribution, may be non-independent, and may exhibit autocorrelation, rendering traditional analytical methods inadequate to effectively analyze the data (Zuur et al., 2009). Unfortunately, adapted statistical methods that account for the complexity of LTEs data are not often used, which means that a large amount of valuable data is not fully utilized, potentially leading to missed opportunities to uncover important relationships and insights into long-term soil management.

1.4 OBJECTIVES OF THE THESIS

We saw that crop fertilization is crucial for maintaining crop production and feeding the world's population. However, conventional mineral fertilizers face serious challenges such as limited reserves, GHGs emissions, and energy consumption, and it is imperative to find alternatives. Among the numerous candidates for mineral fertilizers substitutes, OW have been widely used and have well-established effects. LTEs are the most relevant and reliable way to evaluate the effects of OW application, since these can last for several years and may become measurable after years. Although a considerable number of LTEs were focused on fertilization, there remain questions about the variability of effects depending on the type of OW, the underlying causes of these effects, and which effects are most prominent when all factors are considered. Additionally, there is uncertainty about whether these effects are still significant when OW is used in the way farmers typically use them or in calcareous which is not depleted in OM at the beginning.

Thus, the objective of the thesis will be to comprehensively characterize some of the agronomic effects and environmental impacts of repeated applications of different OW to soils and their determinants. The following research questions were used as a

guiding thread in this work:

- How does the crop yield change over time with repeated OW applications, with partial or full substitution of mineral fertilizers? What is the effect of OW type and rate? What are the main factors that contribute to yield variation (by soil nutrient reserves build up or direct nutrient inputs)?
- How repeated applications of different OW affect the soil biological, physical and chemical properties? What are the relationships and interactions between them? Whether these impacts are the same across sites or not? What are the soil properties that mainly react to OW applications?
- Do different types of OW have different and significant impacts on the soil physico-chemical properties, fertilizer savings and crop yield in a LTE conducted according to usual farmer practices on a calcosol?

In detail,

Chapter II gives an overview of the two LTEs used in this work and the network to which they belong. Chapters III, IV, and V are the core of the work presenting results to answer the research questions listed above.

Chapter III used both the PRO'spective and Qualiagro sites (i) to investigate the temporal dynamics of crops yields after repeated applications of various types of OW, in partial or complete substitution of mineral fertilizers and (ii) to disentangle the effects of the direct application of available nutrients by OW and of the improved soil fertility, for maize the year of OW application and for wheat the year after.

Chapter IV used both the PRO'spective and Qualiagro sites to (i) determine the soil biological, physical, and chemical soil properties changes after long-term and repeated OW applications; (ii) summarize these changes in a global assessment of the soil quality changes under different OW applications, and (iii) identify the link between soil properties changes and OW inputs.

Chapter V based on the LTE site PRO'spective explores the effects of repeated applications of different OW on (1) crop yield and N, P, and K contents; (ii) soil chemical fertility (C, N, P, and K contents); and (iii) soil mineral N supply and risk of N losses.

Chapter VI summarizes the most salient results and discusses further implications for future research.

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Chapter II

Experimental Sites

2 EXPERIMENTAL SITES

2.1 THE SOERE-PRO NETWORK

The French research observatory SOERE PRO is a long-term field experiments network dedicated to the study of the risk and benefits associated with organic wastes use. SOERE-PRO is a network of five main instrumented sites (QualiAgro in Paris area, PROspective in Alsace, EFELE in Brittany, La Mare in Reunion island and SOERE PRO-Sénégal in Senegal) and four associated sites (La Bouzule in Lorraine, Couhins in Aquitaine, Nouzilly in Centre-Val-de-Loire, Gampéla in Burkina Faso) (Figure 2-1). The SOERE-PRO network forms part from the research infrastructure ANAEE-France, which provides services to study the ecosystems (<https://www.anaee-france.fr/>).



Figure 2-1 Installation of SOERE PRO sites. (SOERE PRO, 2015).

The SOERE PRO network involves different research institutes (INRAE, CIRAD, IRD) and collaborations with concerned professional partners. Completed data and information from each site, include: field-experiment management information and weather data; OW data; soil data; plant data and so on. These data were used to evaluate the effect of OW; simulate the long-term consequence of regular OWP application and test various alternative scenarios.

Thus, the objectives of the SOERE PRO network are:

- To determine and simulate how OW recycling modifies organic matter (OM) dynamic in soils, biogeochemical cycles related with OM, and other biogeochemical cycles of major nutrients (P, K...);
- quantify the environmental impacts related to gas emission (NH₃, greenhouse

gas (GHGs), volatile organic compounds (VOCs)) and assessment of emission factors;

- to parameterize models simulating the biogeochemical cycles in agrosystems receiving OW;
- to study the effects of recycling of OW on soil physical, chemical, and biological properties and functioning;
- to identify and rank the parameters that condition contaminant behavior in the agrosystems and their evolution with repeated OW applications;
- to determine the ecotoxicological risks associated with OW repeated applications.

In this thesis, we focus on the two oldest and ongoing LTE: PROspective and QualiAgro.

2.2 SITE PROSPECTIVE

The LTE PROspective were set up in 2000 by INRAE in collaboration with the Syndicat Mixte pour le Recyclage Agricole du Haut-Rhin (SMRA68) and l'Association pour la Relance Agronomique en Alsace (ARAA). This experiment was initiated after the implementation of regulations regarding the spreading of sludge and the standardization of composts, thereby highlighting the effect of OW on local agricultural practices. The initial objectives were to characterize the agronomic value and the environmental impacts of OW, particularly the effect of the composting process. In detail, the objectives of the PROspective were:

- To assess the agronomic benefits of OW, including their impact on crop yields, soil organic matter, biodiversity, fertility, and potential as a fertilizer substitute.
- To determine the long-term environmental impacts of OW, such as the fate of contaminants, trace elements, and organic trace compounds.
- To evaluate the impact of OW application on the quality of water that percolates through the soils.

The objectives of the study have shifted within the framework of a circular economy since 2015. The new goal is to examine the feasibility of combining original OW with another form of OW (digestate), with a particular emphasis on the potential to completely replace mineral fertilizers with OW.

The experiment field is located at the INRAe Colmar Experimental Centre, northeastern France (48°03' N, 7°19' E, altitude 200 m). The climate is semicontinental (Cfb in Köppen-Geiger classification), with a mean annual precipitation of 559 mm received mostly between May and October and an average annual air temperature of 11.3 °C. This plot was fertilized with potassium slag (approximately 150 kg K₂O/ha per year) from 1956 to 1980, as well as regular additions of phosphate fertilizers and organic matter, prior to the initiation of the trial. This was a common practice for the plot and

reflected the typical fertilization regime in use. The soil profile presents the following sequence of horizons (Isch, 2016):

- 0–30 cm, corresponds to the horizon subjected to tillage and comprises a mixture of organic and mineral matter which forms a stable complex and presents a lumpy to polyhedral structure. Compact but not friable with many roots.
- 30-60 cm: a calcareous structural S horizon. Not very compact while not friable with subangular polyhedral structure (30-50 mm) and numerous roots. The clay-humus complex is still saturated with Calcium ion.
- 60-120 cm: gradual transition between the structural S horizon and a calcareous C horizon, not very compact and friable while very calcareous and many pseudomycelia.
- 120-160 cm: sandy loam to loamy sand with numerous pseudomycelia, calcareous concretions and a few pebbles (10 to 20%).
- 160-200 cm: stony sand from the Fecht alluvium with many pebbles (60 to 80%), particulate structure, not very compact but very friable. Without roots and calcium.

Prior to the initiation of the experiment, the initial soil conditions were characterized by the following parameters (Table 2-1). The initial content in organic C; Olsen P₂O₅ and exchangeable K₂O were relatively high (14.3, 0.07 and 0.32 g/kg, respectively). The soil pH equal to 8.3 indicated an alkaline soil.

Table 2-1 Soil initial conditions in the plowed layer (0-28 cm) at site PROspective (mean value ± standard deviation).

Date (year.month)	pH water	CEC (cmol+/kg)	Organic C (g/kg)	Total N (g/kg)	Olsen-P₂O₅ (g/kg)	Exchangeable-K₂O (g/kg)
2000.09	8.3±0.0	16.1±0.9	14.3±0.9	1.4±0.1	0.07±0.01	0.32±0.03

The 2.24 ha experiment field is composed of 5 blocks of 12 plots (10 m × 45 m for each plot). All plots were separated by 10 m wide buffer strips to avoid cross contamination. There were 4 experiments arranged side by side, including two levels of N supplementation subtest with complete randomized block design, one bare fallow subtest without replicate and one subtest cultivated without OW and with rotation of mineral N supplementation (Figure 2-2). It is important to note that the mineral supplementation was adjusted based on the nitrogen equivalent coefficient of each OW and the calculation of the predicted nitrogen balance in the soil. Each subtest includes five OW treatments (1) a dehydrated urban sewage sludge (SLU); (2) a compost of green waste and SLU (GWS); (3) a biowaste compost (BIO); (4) a farmyard manure (FYM); and (5) a farmyard manure compost (FYMC), and (6) a control without any OW application (CON).

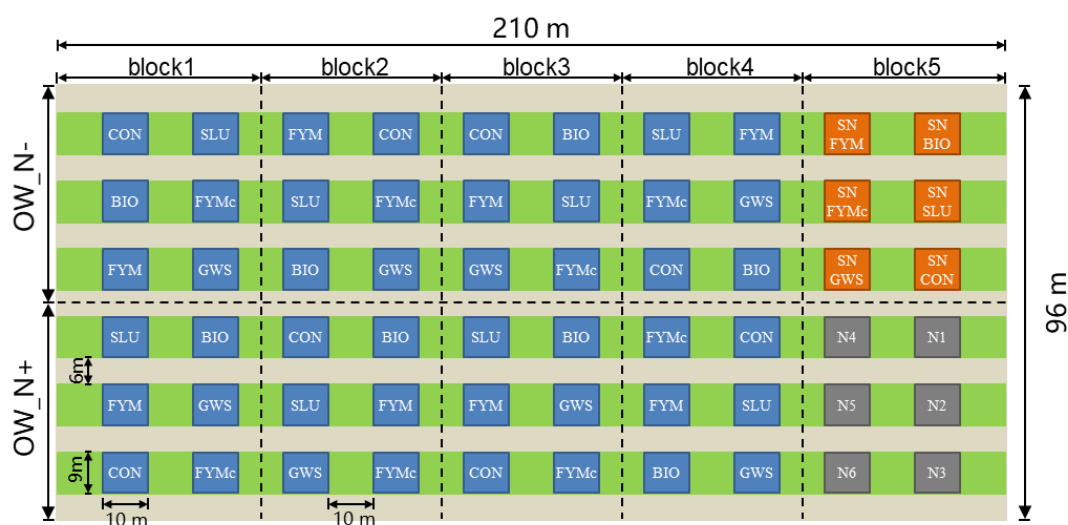


Figure 2-2 The PROspective field experiment to study the effect of organic waste applications. In blue: cultivated experiment with different OW and with (OW_N+) or without (OW_N-) mineral N supplementation, In darkgreen: bare soil experiment with different OW. In grey: cultivated experiment without OW and different level of N fertilization.

From 2000 to 2014, the OW application rate was set to 170 kg N ha⁻¹ (Table 2-2). Owing to the characteristics of the different OW, this application rate resulted in different C, P and K rate, ranging for example from 1.1 t C/ha for SLU to 2.7 t C/ha for FYM, or from 82 kg P₂O₅/ha for BIO to 218 kg P₂O₅/ha for GWS. After 2014, a modified fertilization strategy was adopted for the no mineral N supplementation treatments (OW_N-), aiming to attain comparable yields to the treatments with mineral N supplementation (OW_N+). Consequently, the amounts of OW in the OW_N- treatments were adjusted for each application and treatment, taking into account the nitrogen requirements of the spring crop as well as the specific characteristics of each OW. More information about OW and the application strategy will be described in detail in the Chapter III, Chapter IV and Chapter V.

Table 2-2 Mean applied organic waste (OW) over the 2000-2013 period at site PROspective (mean value ± standard deviation).

OW	Organic C (t/ha)	Total N (kg/ha)	P ₂ O ₅ (kg/ha)	K (kg/ha)
SLU	1.0±0.1	161±25	178±38	16±4
GWS	2.2±0.8	178±41	218±39	93±46
BIO	2.0±0.3	161±17	82±10	175±24
FYM	2.7±0.4	185±57	104±31	262±69
FYMC	2.1±0.3	154±18	104±21	238±48

The crop succession in PROspective was a maize - winter wheat - sugarbeet - spring barley rotation since 2001 (except for 2003, when maize was sown instead of sugarbeet) (Figure 2-3). This crop succession is based on the diversity and regional representativeness of crops and processed products from harvests, as well as the

diversity of biological models. The experiment is managed according to typical local farming practices, including the sowing dates, the tillage practices, the management of crop residues or the use of pesticides (more information in Chapter III). Since 2014, mustard cover crops before spring crops (sugarbeet and maize) have been used.

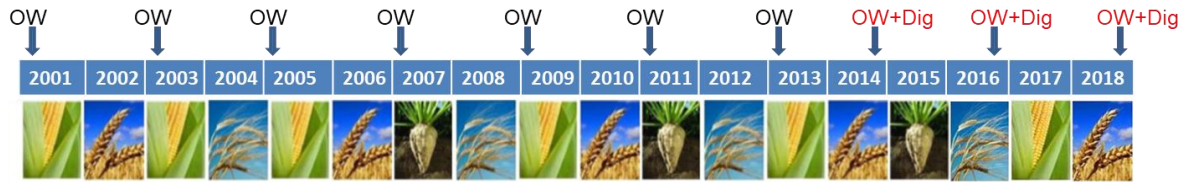


Figure 2-3 Operation of the PROspective experiment, with crop succession and spreading of organic waste (OW).

2.3 SITE QUALIAGRO

The LTE Qualiagro was set up in 1998 by INRAE and Veolia Environment Research and Innovation (VERI). The initial objective of this program is to characterize the agronomic value of urban composts and their environmental impacts compared with a farmyard manure, from a field experiment and laboratory work. Since 2014, the site has been operated with the criteria of organic farming and therefore without chemical inputs or pesticides. The additional objective is to compare the effect of OW application with legumes crop such as alfalfa in organic farming.

The experiment field is located at Feucherolles, northwestern France (48°52' N, 1°57' E, altitude 150 m) (Figure 2-4a). The soil is classified as Luvisol (WRB, 2015), the plowed layer contains on average 17% clay, 78% silt, and 5% sand. The climate is oceanic (Cfb in Köppen-Geiger classification) with a mean annual temperature of 10.8°C and mean rainfall of 594 mm year⁻¹. The soil profile presents the following sequence of horizons (Filipović et al., 2014):

- 0–28 cm, a tilled loamy LA horizon;
- 28–38 cm, a plow pan,
- 38–50 cm: an eluviated silt loam E horizon;
- 50–90 cm: an illuviated silty clay loam BT horizon;
- 90–140 cm: a transition silty clay loam BT/IC horizon;
- 145–200 cm: a silty loam structureless decarbonated loess IC horizon.

Prior to the initiation of the experiment, the initial soil conditions were characterized by the following parameters (Table 2-3). The soil was neutral (pH = 6.9) and the initial soil C content was low (10.5 g/kg).

Table 2-3 Soil initial conditions in the plowed layer (0-28 cm) at site Qualiagro (mean value ± standard deviation).

Date (year.month)	pH water	CEC (cmol+/kg)	Organic C (g/kg)	Total N (g/kg)	Olsen-P ₂ O ₅ (g/kg)	Exchangeable-K ₂ O (g/kg)
1998.09	6.9±0.2	9.5±0.6	10.5±0.7	1.1±0.1	0.08±0.02	0.21±0.03

The experiment is composed of 4 blocks of 10 plots (10 m × 45 m for each plot), separated by 6-m-wide and 25-m-long cultivated bands (Figure 2-4b). Half of the plots in the experiment were assigned to receive a minimal additional mineral N dose (minimal N/Low N), while the other half of the plots received an optimal one (optimal N/high N). Within a half-block, the 5 treatments are randomly distributed, including: (1) a municipal solid waste compost (MSW) made from aerobic composting of residual household waste after selective collection of “clean and dry” packaging; (2) a biowaste compost (BIO) made from the selectively collected fermentable fractions of municipal wastes co-composted with green wastes; (3) a compost of sewage sludge, green wastes, and wood chips (GWS); (4) a farmyard manure (FYM) obtained from a nearby dairy farm; and (5) a control treatment (CTR) that did not receive any organic amendment.

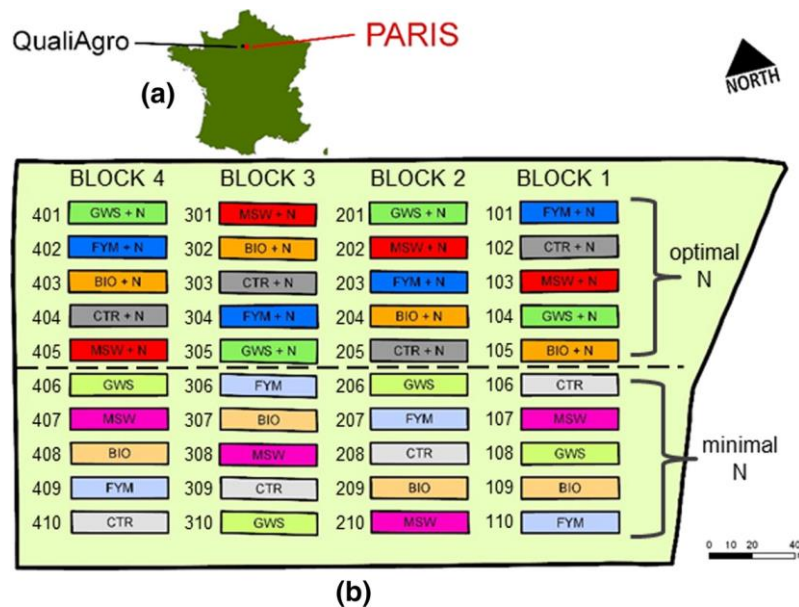


Figure 2-4 The QualiAgro Field experiment to study the effect of organic wastes applications. a Location in the Paris region (France). b 40 plots in four repetition blocks, farmyard manure (FYM), municipal solid waste compost (MSW), biowaste compost (BIO) and a co-compost of green waste and sewage sludge (GWS), control (CTR), minimal additional mineral nitrogen (minimal N/Low N) and optimal one (optimal N/high N). (Gilliot et al., 2021).

Since the implementation of the system, no plot has been fertilized with either phosphorus or potassium. From 1998 to 2013, the quantities of OW application rate was set to 4 t C ha⁻¹ (Table 2-4). Owing to the characteristics of the different OW, this application rate resulted in different N, P and K rate, ranging for example from 215 kg N/ha for MSW to 380 kg N/ha for GWS, or from 100 kg P₂O₅/ha for MSW to 486 kg P₂O₅/ha for GWS. OW were applied over wheat stubble every second year, which allows to incorporate OW and stubbles within the upper soil layer. Since 2014, In the

subtest optimal N/high N, the application rate of OW was divided by two (2 t C ha^{-1}) while OW application were stopped in the subtest minimal N/low N. Commercial organic fertilizer (AxeN12/AxeNP, made of slaughterhouse residues) were introduced in the subtest optimal N/high N. More information about OW application will be described in detail in the Chapter III and Chapter IV.

Table 2-4 Mean applied organic waste (OW) over the 1998-2013 period at site Qualiagro (mean value \pm standard deviation).

OW	Organic C (t/ha)	Total N (kg/ha)	P₂O₅ (kg/ha)	K (kg/ha)
GWS	4.4 \pm 1.0	380 \pm 43	486 \pm 138	245 \pm 100
BIO	3.9 \pm 0.7	320 \pm 51	199 \pm 62	407 \pm 103
MSW	3.8 \pm 1.0	215 \pm 76	100 \pm 37	127 \pm 47
FYM	4.1 \pm 0.8	279 \pm 76	167 \pm 48	453 \pm 104

The experimental field site has been cropped with a wheat-maize rotation since the beginning of the experiment, without intermediate crop for simplification. Regular ploughing (0-28 cm) occurred every year in October or November and weed and pest control were achieved through the use of pesticides. Since 2014, the experiment changed to organic farming, therefore mineral N and pesticide uses have been stopped and all crop residues have been returned to the soil. In the high N subtest, maize was planted in 2014, barley in 2015, rye in 2016, barley in 2017, maize in 2018 and wheat in 2019, while in the low N subtest, maize was planted in 2014, barley in 2015, alfalfa in 2016, alfalfa in 2017, maize in 2018 and wheat in 2019 (Figure 2-5).

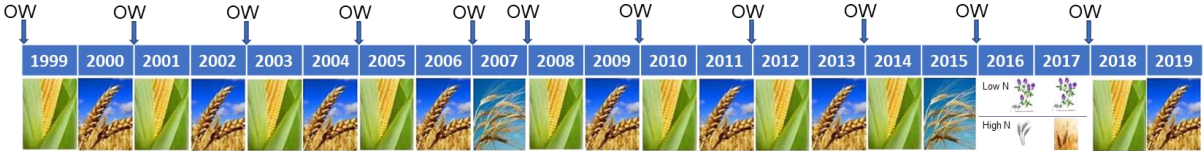


Figure 2-5 The crop succession of the Qualiagro experiment, with crops and spreading of organic waste.

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Chapter III

Substitution of mineral N fertilizers with organic wastes in two long term field experiments: dynamics and drivers of crop yields

This chapter is written as a research paper to be submitted in an international peer-reviewed journal (e.g., *Journal of Plant Nutrition and Soil Science*)

3 SUBSTITUTION OF MINERAL N FERTILIZERS WITH ORGANIC WASTES IN TWO LONG TERM FIELD EXPERIMENTS: DYNAMICS AND DRIVERS OF CROP YIELDS

ABSTRACT

Organic wastes (OW) are rich in nutrients and their recycling into agriculture can substitute chemical fertilizers. The extent of this substitution (partial with complementation with mineral fertilizer or full with only OW applied as fertilizer), the type, rate and period of application of OW, as well as the crop type, may condition the crop productivity. The temporal dynamics of crop productivity after repeated applications of OW is also questioned. Thus, two French long-term field experiments (QualiAgro and PROspective, started in 1998 and 2000, respectively) were used to evaluate the effect of repeated OW applications on crop yield dynamics and investigate the potential driving factors affecting crop yields. A wide range of OW were applied: urban sewage sludge (SLU), green waste and SLU compost (GWS), biowaste compost (BIO), municipal solid waste compost (MSW), farmyard manure (FYM), and composted FYM (FYMC). The OW were applied every two years, maize was planted during the year following the application and wheat during the second year. The results indicated that at the QualiAgro site, a combination of OW and high mineral N treatments resulted in higher maize and wheat yields compared to the mineral N control, while the combination of OW and low mineral N reached the same maize and wheat yield as the mineral N control after 3 and 6 applications of OW, respectively. At the PROspective site, partially substituting mineral fertilizer with OW maintained maize yields but decreased wheat yields, while full substitution led to a decrease in both maize and wheat yields compared to the mineral N control. Results from the gradient boosting model (GBM) showed that soil total N rather than mineral N input was the primary driver of the relative maize yield, while mineral N fertilizer input was more critical for wheat during the second year. We conclude that the joined use of OW and mineral fertilizers is the better way to maintain high yields and soil fertility. We further suggest that OW full substitution of mineral fertilizer may need to apply OW more frequently to meet the crop demands, and/or to use OW with higher N availability like digestates.

KEYWORDS

Maize productivity; Wheat productivity; Long-term field experiment; Organic waste recycling; Fertilizer substitution

3.1 INTRODUCTION

To support ever-increasing population and dietary changes, promoting crop productivity on limited cultivable land has become a great challenge in modern agriculture (Ibarrola-Rivas et al., 2017). Mineral fertilizers enable farmers to feed a booming global population, and it is estimated that about 44% of the world population is supported by mineral N fertilizers (Erisman et al., 2008). However, mineral fertilizers are basically non-renewable resources that require extensive use of fossil fuel resources. It was reported that production of one metric ton of N fertilizer by the Haber-Bosch process requires 873 m³ of natural gas (Vance, 2001). With continuously depleting resources of fossil fuels, there is a need to explore bio-based alternatives to fossil-based fertilizers. Moreover, excessive mineral fertilizer has been applied in order to improve crop production, hence leading to a series of adverse environmental consequences such as land degradation (Ayub et al., 2020) and water eutrophication (Foucher et al., 2020). Therefore, how to enhance crop productivity with low environmental impact is one of the most urgent issues in agriculture nowadays.

Recirculation of organic wastes (OW) rich in nutrients has been considered as an important way to enhance soil fertility, crop productivity, and sustainability of production system. OW applications could directly promote plant growth by supplementing more nutrients and also indirectly affect crop yields by enhancing soil fertility, such as improved soil structure stability (Annabi et al., 2011), water holding capacity (Celik et al., 2004), and nutrients availability (Chalhoub et al., 2013). Previous studies have investigated the effect of recycling OW on crop yields (Chen et al., 2022; Diacono and Montemurro, 2010; Edmeades, 2003; Rasool et al., 2008; Zavattaro et al., 2017), and overall results reported that partial substitution of mineral fertilizers by OW maintained or increased crop yield whereas full substitution might decrease yields (Zavattaro et al., 2017; Zhang et al., 2020). Moreover, the effect of OW on crop yields also depends on crop types (Chen et al., 2018), OW types (Chen et al., 2022), soil and climates conditions (Du et al., 2020) as well as management practices (Wang et al., 2020).

In addition, the application of OW in field has residual effects that last for years, and these effects would benefit to soil quality and succeeding crops (Reeve et al., 2012). Depending on the climate, soil type, and OW types, these residual effects on crops could last 1 to 6 years after OW application (Reeve et al., 2012). Thus, it is usual for farmers to apply OW every several years in the fields. The repeated applications of OW could thus progressively increase the crop yields after an initial decrease in case of a full substitution of mineral fertilizers (Maltas et al., 2018).

The objectives of this study were (i) to investigate the temporal dynamics of crop yields after repeated applications of various types of OW, in partial or full substitution of mineral fertilizers, and (ii) to disentangle the effects of the direct application of available nutrients by OW and of the improved soil fertility. We focused on the yield of maize

the year of OW application and of wheat the year after in two French long-term field experiments.

3.2 MATERIAL AND METHODS

3.2.1 Site description

This study was conducted on two French long-term field experiments belonging to the SOERE-PRO-network (https://www6.inra.fr/qualiagro_eng/Nos-partenaires/The-SOERE-PRO-network): QualiAgro (QUA, started in 1998) and PROspective (COL, started in 2001). At both sites, OW has been applied every two years since the beginning, but the sites differ in soil properties, climate, crop succession, and OW types and rates. In this study, we focused on the two common crops (wheat and maize).

3.2.1.1 Field experiment QualiAgro

The QualiAgro experimental site (48°90'N and 1°95'E) is located within the Plateau des Alluets (Yvelines, France). The soil is classified as horticultural glossic Luvisol (IUSS Working Group WRB, 2014), and the plowed layer contains on average 17% clay, 78% silt and 5% sand. The climate is oceanic with mean annual precipitation of 650 mm and a mean annual temperature of 10.8°C.

The design of the experiment was already described in previous studies (Cambier et al., 2019; Paradelo et al., 2019). In brief, the 6-ha field experiment design was a randomized complete block consisting of 4 replicates of 5 different OW modalities: (1) a municipal solid waste compost (MSW); (2) a biowaste compost (BIO) made from the source-separated fermentable fraction of municipal solid waste co-composted with green wastes; (3) a compost of sewage sludge, green wastes and wood chips (GWS); (4) a farmyard manure (FYM) obtained from a dairy cow farm, and (5) a control without organic amendment (CON). In addition, two levels of mineral N fertilization were also set: (1) an optimal mineral N fertilization level (QUA_N), which aimed to obtain the same optimal maize and wheat for all treatments (except for maize crop in the OW treatments since 2008 in which mineral N fertilization was stopped because of increased mineral N availability); (2) a low mineral N fertilization level (QUA_LEG), where no mineral N fertilization for maize and approximately half rate of mineral N for wheat in comparison to QUA_N were applied. The detailed amounts of OW applied during the experiment are shown in Table S1.

From 1998 to 2013, OW was applied every second year at a rate of 4 t C ha⁻¹ in September on wheat stubbles. The field was subjected to annual plowing to a depth of 28 cm, and pesticides were used to control weeds and pests. The crop rotation was grain maize - winter wheat, except in 2007 when a winter barley was inserted. Wheat straw was exported whereas the maize residues were incorporated into the topsoil layer. Maize harvest takes place in October (except in September for 2003) and wheat harvest takes place in July. Since 2014, mineral N and pesticide uses have been stopped and all

crop residues have been returned to the soil. In the QUA_N experiment, the half dose of OW (2 t C ha^{-1}) was applied while OW application was stopped in QUA_LEG. Winter barley – rye - spring barley – grain maize – winter wheat has been cropped in the QUA_N experiment while rye and spring barley were replaced by alfalfa in the QUA_LEG experiment in 2016 and 2017.

3.2.1.2 Field experiment PROspective

The PROspective experiment is located at the Colmar Experimental Centre of the French National Research Institute for Agriculture, Food and Environment (INRAE), northeastern France ($48^{\circ}03'N$, $7^{\circ}19'E$, altitude 200 m). The soil presents a silty loam texture (21% clay before decarbonatation, 70% silt, and 9% sand), and is classified as Calcosol (IUSS Working Group WRB, 2015) ($13\% \text{ CaCO}_3$). The crop succession established in 2001 was a grain maize - winter wheat - sugarbeet - spring barley rotation (except for 2003, when sugarbeet was replaced by maize). Regular plowing occurred every year at 28 cm. Pesticides are used in the field to control weeds, insect infestation, and pathogens. After 2014, mustard was planted as cover crops before maize and sugarbeet. More details information about the trial design is available in Chen et al. (2022) (Chapter V).

The 2-ha experimental field was used to compare 4 replicates of 5 OW modalities: (1) a dehydrated urban sewage sludge (SLU) derived from a local wastewater treatment plant; (2) a compost of green waste and SLU (GWS) derived from the abovementioned SLU; (3) a biowaste compost (BIO) made from the home-sorted organic fraction of municipal solid waste co-composted with green waste; (4) a farmyard manure (FYM) obtained from a dairy farm; and (5) a farmyard manure compost (FYMC), resulting from 2 months of composting in the open air of the abovementioned farmyard manure, and (6) a control without any OW application (CON). Meanwhile, two levels of N supplementation were tested: (1) no mineral N supplementation (COL_N-), with no mineral N applied during the experiment; and (2) optimal mineral N supplementation (COL_N+), where mineral N application was adjusted depending on the available N stocks in the end of winter and aimed to obtain economically optimal yields. The detailed amount of OW applied during the experiment are shown in Table S.2, S.3, and S.4.

From 2001 to 2013, OW was applied every second year in early spring before maize or sugarbeet sowing with an amount of approximately 170 kg N ha^{-1} . All crop residues were returned to soils until 2013. After 2014, the OW application period changed to summer before cover crop sowing. In the COL_N- experiment, the fertilization strategy was changed to reach optimal yields and therefore the quantity of OW applied was calculated based on the N requirements of the following spring crop (in terms of available N). In addition, a raw digestate of biowaste was also applied every year if needed to supply the additional required amount of available N. The wheat crop residues were exported after 2013, but maize residues were still returned.

3.2.2 Sampling and analysis

Soil samples were collected from the cultivated horizon (0–28 cm) at both sites and analyzed before the beginning of the experiment and every two years after crop harvest and before OW application. Collected samples were analyzed with certified standard methods at INRAE's National Soil Analysis Laboratory (LAS) (<https://www6.hautsdefrance.inrae.fr/las>). Soil pH was measured in a 1:5 soil: liquid suspension (v/v) (NF ISO 10390). Total N was determined by dry combustion (NF ISO 13878). The availability of potassium (AK) was determined by measurement of exchangeable K extracted and analyzed by ICP-AES. The availability of phosphorous (AP) was determined through the measurement of extractable Olsen-P using sodium bicarbonate extraction (NF ISO 11263). Every 2 or 3 years, the soil core method was used to determine soil bulk density (Blake and Hartge, 1986).

Plants were sampled manually every year immediately before mechanical harvest and the grain yield was obtained. Grain and crop residues were separated manually and oven-dried at 103 °C to constant weight for the analysis of the dry matter.

In order to compare the two sites and different climatic years, we computed each year the relative crop yield (RY) for the OW treatments and the low N control treatments, relative to the corresponding mineral N control (QUA_N_CON, COL_N+_CON):

$$RY (\%) = Yield_{OW} / Yield_{mineral_N_control} \times 100 \text{ (Equation 3-1)}$$

Where $Yield_{OW}$ is the crop yield of the OW treatments and $Yield_{mineral_N_control}$ is the crop yield in the corresponding mineral N control treatment.

3.2.3 Gradient boosting model

A gradient boosting model (GBM) was performed to reveal the relative influence of OW inputs and soil characteristics on relative crop yields. Soil pH, Δ soil N, Δ soil AP, Δ soil AK, total N input from OW, mineral N input from OW, P input from OW, K input from OW and additional mineral N input from fertilizers were set as predictor variables for relative crop yields, where Δ soil N, Δ soil AP, and Δ soil AK were calculated from the differences of total N, AP, and AK in each OW treatment and the corresponding mineral N control (COL_N+ and QUA_N). Detailed data are shown in Figure.S1 and Figure.S2. For this modeling, we focused on the data from 1998 to 2013 at site QualiAgro and 2001 to 2014 for site PROspective to avoid considering the effect of the changes in experiment management (e.g., ceasing of the pesticide use). The 2003 data in COL was also excluded because of an experimental protocol mistake, detailed in Chen et al. (2022).

In GBM modeling, four parameters require to be specified: interaction.depth (the maximum number of nodes per tree), n.trees (the number of gradient boosting iterations), shrinkage (the learning rate), and n.minobsinnode (the minimum number

of observations in the tree's terminal nodes). Here, the `interaction.depth` and `n.minobsinnode` were set from 2 to 10 with an interval of 2. The `n.trees` was set in the range of 100 to 1000 with an interval of 100. Three-degree levels included 0.01, 0.05, and 0.1 were tested to find the optimized shrinkage level. The model with the lowest root means square error (RMSE) was selected as optimal. We evaluated the performance of the calibration results using a 5-fold cross-validation (CV) method. The entire dataset was randomly partitioned into 5 folds, where 4 folds of data were used to build the ensemble-based model, while the remaining folds were used for model validation. The GBM model was implemented using the 'gbm' package in R 4.0.4 (R Core and Team, 2019).

3.2.4 Statistical analysis

All statistical analyzes were achieved with R software (R Core and Team, 2019). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$ level of significance) was used for the separation of means.

3.3 RESULTS AND DISCUSSION

3.3.1 Absolute crop yield evolution in the mineral N control treatments

In the first years of the experiments, very similar yields were measured for both maize and wheat in QUA and COL (Figure 3-1a). The average dry matter maize yield in the high mineral N control treatments in QUA (QUA_N) was 8.8 t DM/ha prior to the management changes in the experiment, while in the mineral N control treatments in COL (COL_N+), it was 9.4 t DM/ha over the entire experimental period. These yields were similar to the mean regional yield obtained by farmers, 9.1 and 9.9 t DM/ha, respectively (Soleilhavoup & Crisan, 2021). After the experimental protocol change in QUA the maize yields showed a dramatic decrease (mean yield equal to 4.1 t DM/ha, i.e., -53% compared to the period before), larger than the usual decrease observed for maize yield after conversion to organic farming (-14%, Seufert et al., 2012).

Wheat yields were high in the mineral N control treatments in both QUA and COL (Figure 3-1b), with average yields over the period equal to 7.2 and 6.6 t DM/ha, respectively (before the changes in experiment management for QUA). These yields were similar to the mean regional yield obtained by farmers, 7.1 and 6.9 t DM/ha, respectively (Soleilhavoup & Crisan, 2021). The only wheat after the experiment change in QUA showed a moderate yield decrease with a yield equal to 5.4 t DM/ha, i.e., -25% compared to the period before, comparable to what is usually observed after the conversion to organic farming (-39%, Seufert et al., 2012).

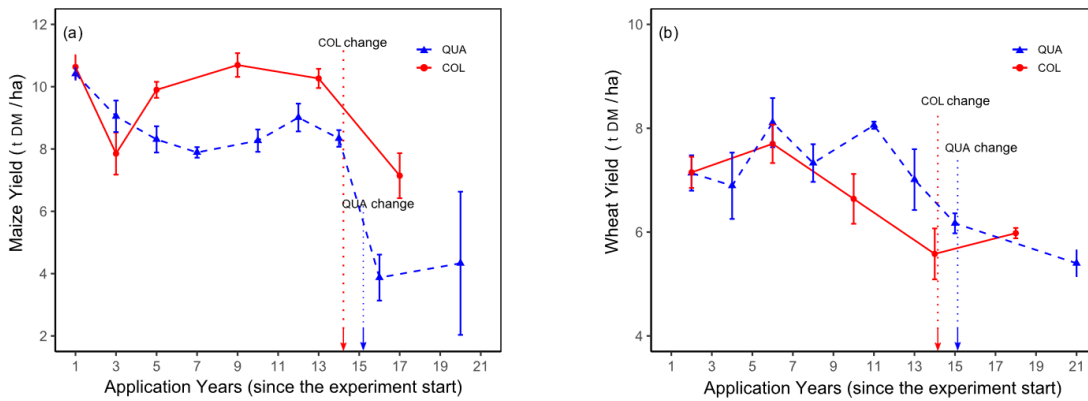


Figure 3-1 Evolution of (a) maize and (b) wheat grain yield in mineral N treatments (COL_N+ and QUA_N). Error bars are the standard deviations (four replicates). The vertical dotted lines represent the changes in the experiment management.

3.3.2 Relative crop yield evolution

In COL, the incorporation of OW with mineral N made it possible to reach the same maize yield as in control with mineral N throughout the period (Figure 3-2a). Without mineral N addition, only SLU in COL_N- allowed to reach the same yield throughout the period. The OW application (except in SLU treatment) in COL_N- maintained the maize yield only in the first years, then the yield subsequently decreased and stabilized at a significantly lower level until the digestate was applied in the last year (Figure 3-2a). When digestate was used, all the treatments reached back the initial yield level.

In QUA_N, the relative maize yields fluctuated from year to year in the treatments receiving OW combined with high mineral N, but generally increased with time and reached significant higher maize yields compared with the control CON_N. In QUA_LEG, the relative yields in the OW treatments were lower than in the mineral N control at the start of the experiment but increased over time and reached a similar level after five years. Then, until the experiment change, the yield slightly fluctuated and reached higher yields depending on the years and OW types. The lowest relative grain yield occurred in CON_LEG, which experienced a downward trend except in the final year (Figure 3-2b).

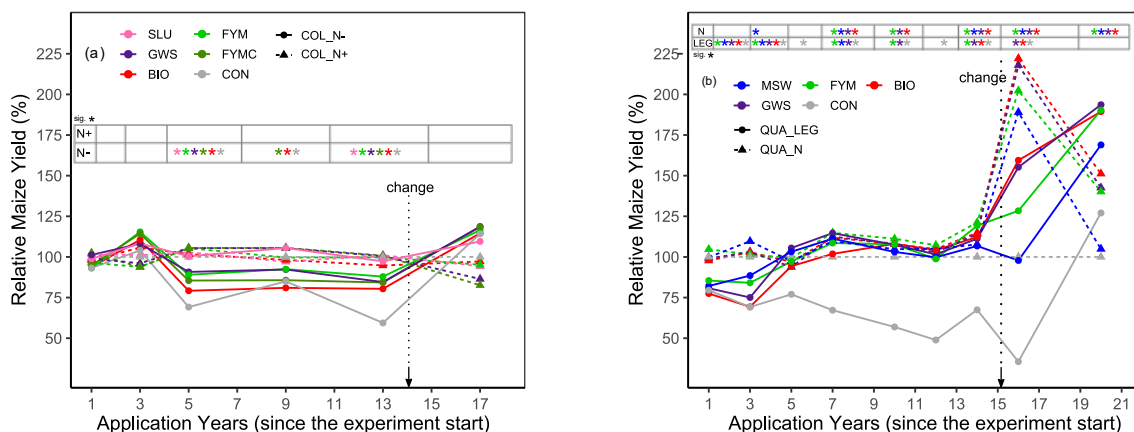


Figure 3-2 Evolution of relative maize grain yields in COL (a) and QUA (b). Asterisks * indicates significant absolute yield difference with the mineral N control treatment (CON_N+ in COL and QUA_N in QUA). For the abbreviations used in this figure, please refer to the List of Abbreviations section.

In COL_N+, the application of OW complemented with mineral N maintained the wheat yield in the first year which then decreased only in the year after, then increased again until reaching the same level as in the mineral N control treatment. The decreases were more pronounced than for maize in the COL_N- experiment and OW without mineral N addition significantly decreased wheat yield until digestate was incorporated after 14 years (Figure 3-3a). In the final year, all the treatments reached the same yields except CON_N- with digestate which obtained a higher yield than in the mineral N control.

The relative wheat yields in QUA_N exhibited slight fluctuations but with an overall upward trend. The OW treatments maintained or even increased the yield with increasing numbers of OW applications until the experiment changed. For QUA_LEG, the OW treatments markedly decreased wheat yield at the beginning. Then, the wheat yields progressively increased with repeated OW applications and ultimately were equal to those of the mineral N control treatment after 13 years (Figure 3-3b). In the last year of the experiment, after 2 years of alfalfa, all treatments in QUA_LEG including the control obtained significantly higher yields compared with QUA_N.

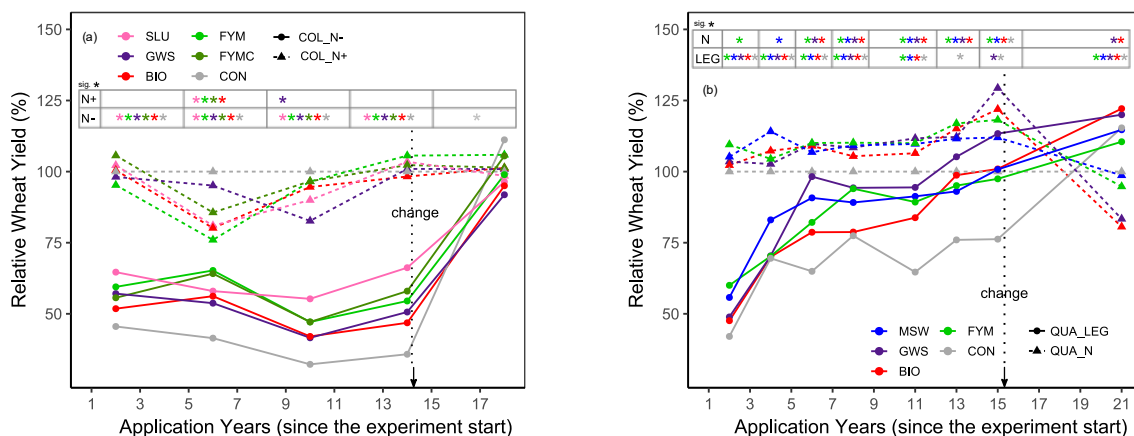


Figure 3-3 Evolution of the relative wheat grain yields in COL (a) and QUA (b). Asterisks * indicates significant absolute yield difference with the mineral N control treatment (CON_N+ in COL and QUA_N in QUA). For the abbreviations used in this figure, please refer to the List of Abbreviations section.

We found that partial substitution of mineral nitrogen fertilizer by OW had the ability to maintain or even increase the maize crop yields the year of OW application, which is consistent with previous studies (Rasool et al., 2008; Zavattaro et al., 2017). However, in COL_N+ where organic inputs were low and the mineral N fertilization decreased depending on available soil mineral N stocks at the end of winter, OW applied every 2 years was not sufficient to maintain wheat yields the year after OW application. In QUA, where a higher amount of OW was applied and mineral N fertilization of wheat was equal among all treatments in QUA_N, we found that the yield increased over time for wheat the second-year after OW application and became higher than in the control mineral N treatment. Similar results were obtained by Maltas et al. (2018). Moreover, in QUA_LEG where mineral N fertilization was decreased, the wheat yields progressively increased with OW applications and finally reached the same yield as in the control mineral N treatment. This showed that at high rates, OW allowed a partial substitution of mineral N fertilizers even two years after application without decreasing crop yields. This phenomenon is attributed to the residual effect of OW applications on the succeeding crops. Generally, the residual effect of OW can be classified into two categories: nutritive effect including the carry-over of nutrients especially with slowly mineralization organic N in OW (Cela et al., 2011; Schröder et al., 2005), and also non-nutritive effects including improvement of soil biophysical and physico-chemical properties (e.g., microbial activities, soil pH, bulk density and water holding capacity) (Bhogal et al., 2011; Eghball et al., 2004). Indraratne et al. (2009) demonstrated that the soil properties such as soil organic matter, N content, phosphorus content and electrical conductivity were still significantly higher than in control after 16 years since OW application ceased.

The full substitution of mineral N by OW allowed to reach the same (or higher) maize yield after 5 years of repeated OW applications in QUA with high OW rates. In contrast in COL_N- with lower OW rates, this full substitution implied a yield decrease, except with SLU, thanks to higher mineral N content compared with other OW (Table.S2) and

more easily degradable organic matter (Zavattaro et al., 2017). Some studies reported that a full mineral N substitution with OW did not compromise yield. For example, Geng et al. (2019) reported that 240 kg ha⁻¹ mineral N replaced by equal cow manure and chicken manure maintained spring maize yield. However, it is worth noticing that the effects of OW applications were highly depending on the quantity, and we highlighted that the OW rate applied in COL at 170 kg N ha⁻¹ followed the European Union “nitrates” directive (Directive 91/676/EEC). This indicated that at OW regulatory rates, the full substitution of mineral N could possibly decrease crop yields compared with mineral N fertilization. Moreover, the full replacement of mineral fertilizers by OW in COL_N- did not maintain crop yields the second year after OW application for wheat.

In addition, we also observed that the digestate was likely to enhance crop yields, not only compared to other OW but also compared to mineral fertilization. This finding is consistent with other studies reporting that a mineral N full substitution with digestate was able to maintain or even increase grain yield (Bańóg et al., 2020; Garfí et al., 2011; Šimon et al., 2015). As a result of the digestion process, digestate was characterized by higher ammonia content compared to untreated OW. Previous studies demonstrated that the mineralization rate of N in digestate is also higher than in other OW and similar to that of urea (Tambone and Adani, 2017). Once applied in the soil matrix, mineral N from the digestate is rapidly and highly available for plant growth and is comparable to mineral fertilizer (Albuquerque et al., 2012). Finally, our findings reveal that discontinuing the OW application and planting alfalfa led to an increase in the yield of both maize and wheat in QUA_LEG. The improvement in yield can be attributed to the N fixation by alfalfa, which enhances the N supplies to subsequent crops in rotation (Berzsenyi et al., 2000). Furthermore, the presence of alfalfa may reduce the abundance of genes associated with denitrification, thus potentially reducing soil N loss (Samaddar et al., 2021). Previous research has shown that alfalfa has the capacity to provide sufficient N to a maize crop in the first year of rotation (Yost et al., 2013) and in some instances, even to the second-year crop (Yost et al., 2014). Additionally, alfalfa may also help to reduce weed pressure (Tautges et al., 2017).

3.3.3 Driving factors of relative yield

The GBM explained well the variability of relative maize and wheat yields, with 5-fold CV R² equal to 0.90 and 0.93, respectively (Figure 3-4b and Figure 3-5b). The relative importance of explanatory variables showed that Δ Soil N was the most important factor for relative maize yield (relative importance equal to 25%), followed by mineral N fertilizer input (17%), OWNmin input (13%), and soil pH (9%) (Figure 3-4a). For relative wheat yield, a substantial proportion of the explained variance was due to mineral N input (50%) (Figure 3-5a). The relationship between the top 4 factors and relative crop yields can be visualized in terms of Partial dependence plot. Despite some fluctuations, the trend of relative maize yield was an increase with Δ Soil N, N fertilizer input, and OWNmin input (Figure 3-4c, d, and e). The relative wheat yields also increased with Δ Soil N, N fertilizer input, and OWN input (Figure 3-5c, d, and f). Neutral pH was better

for wheat and corn growth (Figure 3-4f and Figure 3-5e).

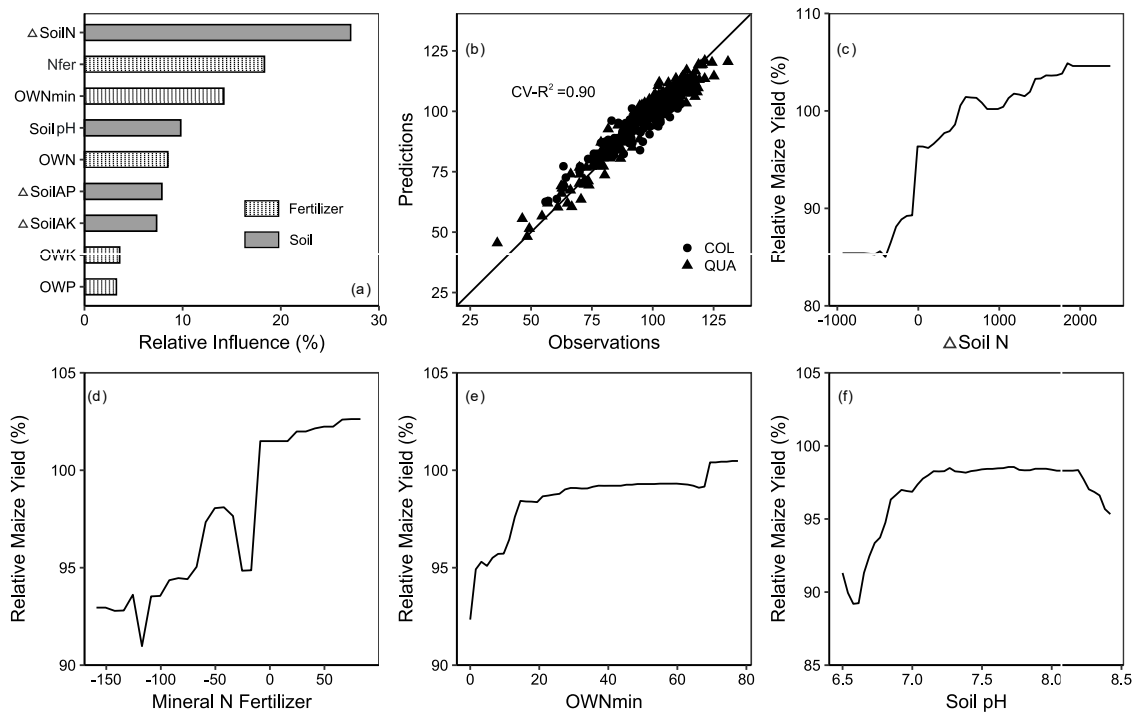


Figure 3-4 Relative influences of explanatory variables for relative maize yield in decreasing order and the marginal effects of the top four influential variables. (Nfer: total N fertilizer input; OWN: total N input from OW; OWmin: total mineral N input from OW; OWP: total P input from OW; OWK: total K input from OW).

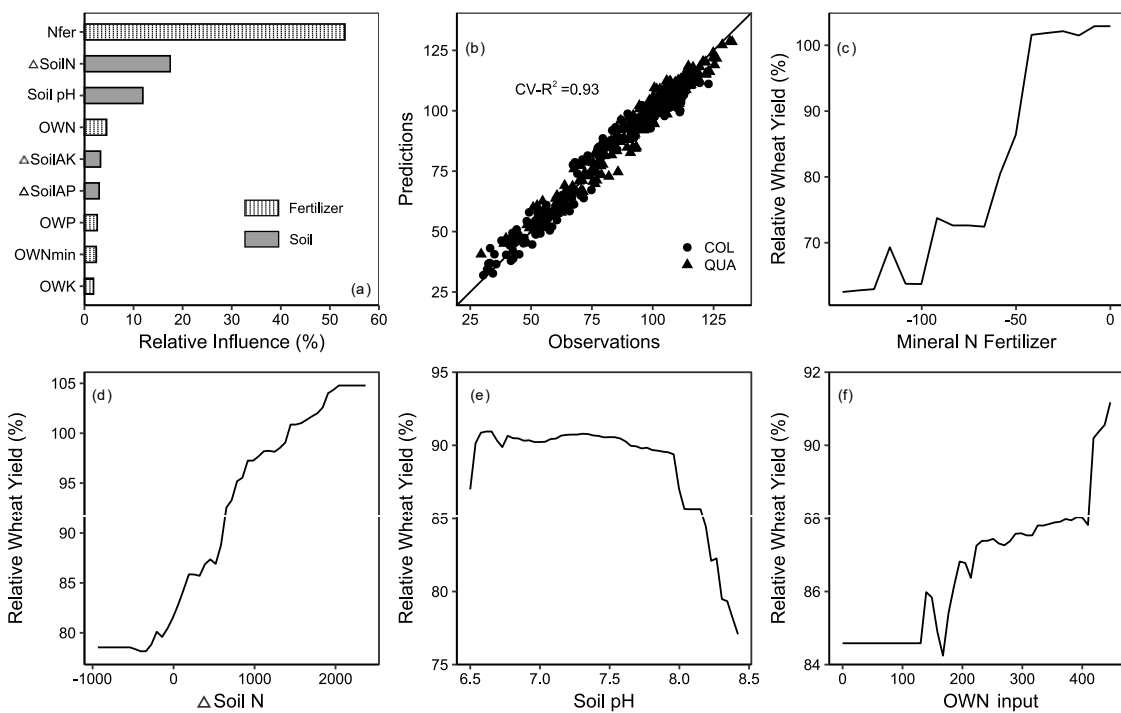


Figure 3-5 Relative influences of explanatory variables for relative wheat yield in decreasing order and the marginal effects of the top four influential variables. (Nfer: total N fertilizer input; OWN: total N input from OW; OWmin: total mineral N input from OW; OWP: total P input from OW; OWK: total K input from OW).

N is the most important plant macronutrient and plays a crucial role in driving crop yields. Maize which was sown directly after OW applications, was mostly affected by soil N content followed by mineral-N fertilizer and mineral-N from OW. Although current research remains controversial as to whether net soil N mineralization rates can meet maize N requirements (Loecke et al., 2012; Osterholz et al., 2017), in our study, soil N still dominated maize N supply and directly determined relative maize yield. Mineral N input from fertilizer and mineral form N in OW was easily taken up by plants, and thence provided more N to meet the shortfall part of maize demand. Wheat was sown the year after maize, more than one year after OW application. Mineral N provided by OW was already taken up by maize. Moreover, the wheat needs nitrogen in the early season and thus only little profit from the increased soil N mineralization which mainly occurred later (Berry et al., 2002). Thus, the wheat relied more on the mineral-N fertilizer input instead of the mineralized N from the soil.

Soil pH plays an important role in driving crop yields. Maize yield showed a clear increasing trend from slightly acidic to neutral, keeping stable for the pH range of 7.1 to 8.2, and decreasing with pH exceeding 8.2. The same trend was also obtained for relative wheat yield which markedly increased until 6.6, remained stable from 6.6 to 8.0, and dramatically declined with pH over 8.0. It was well known that suitable pH could improve soil nutrient availability (Zhao et al., 2011) and use efficiency (Pan et al., 2020), thus increasing crop yields. Previous studies have shown that soil pH between 6.3 and 7.4 was beneficial to maize growth (Sirisuntornlak et al., 2021) and between 6.0 and 7.0 for wheat growth (Vitosh, 1994) which is broadly in line with our results.

However, neither the P and K input nor their contents in available form in soil were markedly related to relative crop yields. This may be attributed to the content of Olsen-P and exchangeable K that exceeded the agronomic critical value in our experiments and therefore resulted in negligible grain yield responses. Based on a 36-year experiment, Colomb et al. (2007) suggested that the agronomic Olsen-P thresholds for maize and wheat were 7.8 mg kg^{-1} and 6.7 mg kg^{-1} . Xi et al. (2016) suggested that indigenous P supply is sufficient to support maize and wheat growth when soil Olsen-P content is higher than 12.3 and 12.8 mg kg^{-1} , respectively. Zhang et al. (2017) carried out a pot experiment and reported that critical exchangeable K concentration of wheat was 91 mg kg^{-1} . Breker et al. (2019) correctly predicted 16 of 25 sites when they set a critical level of exchangeable K for maize at 150 mg kg^{-1} . Although the critical value of soil varies with soil conditions, the mean exchangeable K content and Olsen-P in both QUA and COL were relatively higher than the given level (Figure.S1 and Figure.S2).

3.4 CONCLUSIONS

The partial substitution of mineral nitrogen fertilizer by OW could maintain or even increase crop yields in the long term while the full substitution was insufficient to keep the yield, especially the year after OW application. The result of GBM indicated that the soil total N rather than mineral N input was the main driver to get a similar maize yield

with OW application compared to mineral N, while mineral N input was more important for wheat. Thus, the relative crop yields increased over time with repeated OW applications were mainly due to the increased soil N content. Digestate had the ability to enhance crop yields immediately whereas its build-up effects in the long term still needed to be further studied. Finally, we suggested that OW partial substitution or additional application of digestate were the better ways to keep yields and soil fertility. OW full substitution may need to apply OW more frequently to meet the crop demands, with potential increased nutrient losses and threat to water quality via leaching of excess P and N.

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Chapter IV

Changes, interactions and drivers of soil chemical, physical and biological properties after repeated application of organic wastes in two contrasted long term field experiments in France

This chapter is written as a research paper to be submitted in an international peer-reviewed journal (e.g., Soil Use Management)

4 CHANGES, INTERACTIONS AND DRIVERS OF SOIL CHEMICAL, PHYSICAL AND BIOLOGICAL PROPERTIES AFTER REPEATED APPLICATION OF ORGANIC WASTES IN TWO CONTRASTED LONG TERM FIELD EXPERIMENTS IN FRANCE

ABSTRACT

Recycling organic wastes (OW) is known to influence soil physical, chemical and biological properties. However, there is limited studies comparing the overall effects of different OW at different sites in the long term. Therefore, the objective of this study was to investigate the long-term effects of repeated applications of different OW on soil biological, physical and chemical properties in two French long-term field experiments, QualiAgro and PROspective (20 and 18 years old, respectively). Applied OW were urban sewage sludge (SLU), green waste and SLU compost (GWS), municipal biowaste compost (BIO), municipal solid waste compost (MSW), farmyard manure (FYM), and composted FYM (FYMC). Results showed that soil C, N, Olsen-P, exchangeable K and CEC significantly increased with OW application in almost all the treatments at both sites. Soil pH increased in some treatments in QualiAgro, while it remained nearly stable in the calcareous PROspective experiment. Both active C and stable C increased after OW application while the proportion of stable C decreased. Soil biological properties were less affected compared with physico-chemical properties. OW application increased soil microbial biomass and specific soil enzyme activities while the effect of OW on soil nematode communities varied with the type of OW and sites. The availability of trace elements (TE) in soil also increased with OW application. Correlation analysis showed that almost all soil property changes were correlated. Generalized boosted regression models indicated that the variable exogenous C and nutrient input introduced by OW were the driving factors of the soil properties changes in the different treatments, and thus well explained the larger changes in QualiAgro where the OW application rates were larger. A soil quality index (SQI) summarizing the different soil properties changes showed that OW application significantly increased SQI in most treatments of QualiAgro whereas only FYM and FYMC significantly increased SQI in PROspective where the initial SQI was higher. These results demonstrated that, depending on the type and rate of OW and of the initial soil properties, OW application could change the soil physical, chemical and biological properties hence increasing soil quality.

KEYWORDS

Soil enzyme; Soil nematodes; Long-term field experiment; Soil quality index; Organic wastes

4.1 INTRODUCTION

Organic wastes (OW) application is considered as a valuable strategy to sustain crop productivity by providing nutrients (Chen et al., 2022) and mitigating the environmental impacts of mineral fertilizer use in agriculture. Although there may be some trade-off with OW application, such as increasing trace element contents in soils (Araújo et al., 2019) or the risk of P leaching (Qin et al., 2020), applying OW provides numerous benefits on soil physico-chemical properties, including increasing soil organic matter (SOM) and plant available nutrients (N, P and K) (Diacono and Montemurro, 2010). These benefits largely depend on the type of OW applied (e.g., Chen et al., 2022).

The SOM is crucial for enhancing soil physical properties and fertility, such as soil water holding capacity (Oldfield et al., 2020), soil aggregate stability (Chaney and Swift, 1984) and soil nutrient availabilities for crops (Chalhoub et al., 2013). Depending on their residence time, the SOM could be conceptually divided into active and stable pools (Smith et al., 1997). The active C pool characterized by a lower residence time is known to be highly sensitive to soil management and plays an important role in soil food web, nutrient cycling, soil aggregate stability and agronomic productivity (Almagro et al., 2021; Chenu et al., 2019). The stable C pool has a higher residence time and could contribute to the accumulation of C and to climate change mitigation (Chenu et al., 2019). The effect of OW application on SOM stocks and characteristics differed with the type of OW, field management strategies and soil and climate conditions (Paré et al., 1999; Peltre et al., 2017). A challenge also remained in the qualification and quantification of SOM pools. Recently, Rock-Eval thermal analysis has been proposed for a reliable estimation of the active and stable C pools in soil (Cécillon et al., 2021).

Soil biological indicators (soil microorganisms, enzyme activities, and nematodes) are often found to be more sensitive to changes caused by climatic and anthropogenic factors compared with physico-chemical properties (Bastida et al., 2008). Soil microorganism communities play a critical role in soil nutrient cycles and are directly related to soil biogeochemical processes (Böhme et al., 2005). Soil enzymes are essential for soil biochemical functions and nutrient cycling and facilitate biochemical transformations in soil (Pascual et al., 1998). Soil nematodes occupy key positions in soil food webs and they are the most numerous soil-dwelling multicellular animals (Lazarova et al., 2021). Assessing the soil biometrics dynamic provides important insights into the response of soil quality and ecosystem functions to agricultural practices (Böhme et al., 2005).

Numerous studies from various regions have confirmed that microbial communities, enzymatic activities and soil nematodes are greatly influenced by fertilization management (Chang et al., 2007; Li et al., 2008; Nahar et al., 2006). In general, microbial biomass and enzymatic activities are considerably affected by C and N inputs (Chen et al., 2017; Houot and Chaussod, 1995) and soil nematodes have been highly related to C input especially with belowground C input (Ito et al., 2015; Li et al., 2021). It is worth

noting that the effects of OW applications also changed with their origins and characteristics. For example, it was reported that food waste compost (Lee et al., 2004), pomace (Innangi et al., 2017), farmyard manure (Böhme et al., 2005), and sewage sludge (Criquet and Braud, 2008) increased phosphatase activity whereas municipal solid waste (García-Gil et al., 2000) decreases it.

Despite many studies focusing on specific OW and specific effects of OW in a specific site, few studies have compared the effects of different OW on both chemical, physical, and biological soil properties after long term and repeated applications, at different sites. Few studies also attempted to explain the variables changes in soil properties by the differences of OW characteristics and rates of application.

Thus, the objective of this study was to compare the effects of long-term and repeated applications of different OW on soil biological, physical, and chemical property changes, to highlight the possible interaction between these changes, and to determine the main drivers of these changes related to OW input in two contrasted long-term experiments. Specially, we aimed to (i) determine the soil biological, physical, and chemical soil property changes after long-term and repeated OW application; (ii) summarize these changes in a global assessment of the soil quality changes under different OW applications, and (iii) identify the link between soil property changes and OW inputs. We used data from two contrasted long-term field experiments in France that have regularly received OW.

4.2 MATERIAL AND METHODS

4.2.1 Field experiments

The soil samples used in this study were collected in two French long-term field experiments belonging to the SOERE-PRO network (https://www6.inra.fr/qualiagro_eng/Nos-partenaires/The-SOERE-PRO-network): PROspective (COL, started in 2001), QualiAgro (QUA, started in 1998).

The PROspective experiment is located at Colmar, northeastern France (48°03'N, 7°19'E, altitude 200 m). The soil is classified as Calcosol (WRB, 2015) with 125 g kg⁻¹ calcium carbonate in the plowed layer. The plowed layer contains on average 15% clay (after decarbonation), 78% silt, and 7% sand. The climate is semicontinental (Cfb in Köppen-Geiger classification, Beck et al., 2018), with mean annual temperature of 11.3°C and mean rainfall of 559 mm year⁻¹. The crop succession has been maize (*Zea mays*)-winter wheat (*Triticum aestivum*)-sugarbeet (*Beta vulgaris*)-spring barley (*Hordeum vulgare*) rotation since 2001. The field was subjected to annual plowing to a depth of 28 cm, and pesticides were used to control weeds and pests. Crop residues were returned to the soil. Five different OW have been applied in PROspective experiment: (1) a dehydrated urban sewage sludge (SLU) obtained from the Intercommunal Syndicate for Waste Water Treatment in Colmar and Surroundings (SITEUCE); a co-compost of green waste and the previously mentioned SLU (GWS) by

the forced aeration composting system; (3) a biowaste compost (BIO) made from the home-sorted organic fraction of municipal solid waste co-composted with green waste; (4) a farmyard manure (FYM) obtained from a dairy cow farm which is a mixture of dairy cow feces and urine with cereal straw; and (5) the same farmyard manure composted (FYMC) made from 2 months of composting in the open air on a concrete platform. Two experiments were set up in PROspective: (1) no mineral nitrogen (N) supplementation (COL_N-) and (2) optimal mineral N supplementation (COL_N+). From 2001 to 2013, all OW were applied based on their N content in order to bring the same amount of N (approximately 170 kg N ha⁻¹, maximal annual amount allowed by EU legislation). The OW were applied in early spring before maize or sugarbeet. In 2014 and 2016, the application period changed to summer before the sowing of a catch crop and after the harvest of cereals. In addition, in the COL_N- experiment after 2015, the amount of OW was increased based on the N requirements of the spring crop and a raw biowaste digestate was applied every year if needed to supply the additional required amount of available N to reach the same optimal yield as in the COL_N+ treatment. In addition, the wheat residues were exported in all treatments. Detailed information of PROspective experiment can be found in Chen et al. (2022). The detailed amounts of applied OW and digestate are presented in Table S1 and S2, the characteristics of applied OW and digestate are presented in Table S3.

The QualiAgro experiment is located at Feucherolles, northwestern France (48°52' N, 1°57' E, altitude 150 m). The soil is classified as Luvisol (WRB, 2015), the ploughed layer contains on average 17% clay, 78% silt and 5% sand. The climate is oceanic (Cfb in Köppen-Geiger classification) with a mean annual temperature of 10.8°C and mean rainfall of 594 mm year⁻¹. Four different organic amendments have been applied in QualiAgro experiment: (1) a municipal solid waste compost (MSW); (2) a biowaste compost (BIO) made from the selectively collected fermentable fractions of municipal wastes co-composted with green wastes; (3) a compost of sewage sludge, green wastes and wood chips (GWS); (4) a farmyard manure (FYM) obtained from a dairy cow farm. From 1998 to 2013, the OW applications rate was set to 4 t C ha⁻¹. The crop succession was a wheat–maize rotation. Regular plowing (28 cm) occurred every year and weeds and pest control relied on pesticide use. Wheat straw was exported. Two levels of mineral N fertilization were set in QualiAgro: (1) an optimal mineral N fertilization level (QUA_N); (2) a low mineral N fertilization level (QUA_LEG). Since 2014, mineral N and pesticide uses have been stopped and crop residues returned to soil. In the QUA_N experiment, the application rate of OW was divided by two (2 t C ha⁻¹) while OW application were stopped in the QUA_LEG experiment. The crop succession remains an alternance of maize and other cereals in the QUA_N experiment while alfalfa and fababean were introduced in the crop succession in the QUA_LEG experiment. Detailed information of QualiAgro experiment can be found in Cambier et al. (2019). The detailed amounts of applied OW are presented in Table S4, the characteristics of applied OW are presented in Table S5.

The two sites are thus two long-term experiments with similar duration of OW

application (started in 1998 and 2000 for QUA and COL, respectively), but partly differed in the types and amounts of OW applied, in terms of crop rotation and crop residues management (exported versus returned), and in terms of soil (luvisol versus calcosol) and climate (oceanic versus semicontinental) conditions.

4.2.2 Soil analysis

Soil samples have been collected in 2018 in the frame of the ADEME PROTERR project. For Qualiagro, the samples of soil were collected in April 2018, during the maize growing season. For PROspective, the samples of soil biology analysis were collected in March 2018 during wheat growing season and the samples of soil physico-chemical analysis were collected in July 2018 after wheat harvest. Soil samples were taken from the 0-28 cm horizon (plow layer) at both sites used for soil physico-chemical and trace element analysis. The 0-28 cm horizon (QUA trial) and the 0-20 cm horizon (COL trial) and were used for the biological and Rock-Eval 6 analysis.

4.2.2.1 Soil physico-chemical analysis

After sampling, the soil samples were homogenized by sieving through a 4 mm mesh to ensure uniformity. A portion of samples was air-dried and further grinding (< 200 μm) for physico-chemical analysis according to the standard ISO 11464. Soil physico-chemical analysis and extractable trace elements (TE) were analyzed by soil-testing laboratory of INRAE (LAS, Arras, France). Soil pH was measured in a 1:5 soil:liquid suspension (v/v) (NF ISO 10390). Total C (TC) and N (TN) were determined by dry combustion (NF ISO 10694 & NF ISO 13878). The cation exchange capacity (CEC) was determined using the cobaltihexamine method (NF X 31-130). Available P (AP) was estimated by sodium bicarbonate extraction (NF ISO 11263), as proposed by Olsen (Olsen, 1954). Exchangeable K (EK) was analyzed by ICP-AES. Extractable TE were extracted with 0.05 mol/l EDTA (Cd, Cr, Cu, Ni, Pb, and Zn) as described by Quevauviller (1998). In this study, extractable trace metals were analyzed instead of total TE because they are more closely related to issues of soil quality and toxicity (Gupta and Sinha, 2007).

4.2.2.2 Thermal stability analysis: Rock-Eval 6

The thermal analysis of the samples was performed with a Rock-Eval® 6 (Vinci Technologies, France). Detailed analytical processes and protocols are described in Cécillon et al. (2021). In brief, 30–70 mg of powdered samples were exposed to two consecutive thermal treatments. The first phases of pyrolysis was carried out from 200 to 650 °C with thermal ramping rate of 30 °C·min⁻¹ under N₂ atmosphere, and the second oxidation was carried out from 300 to 850 °C with thermal ramping rate of 20 °C·min⁻¹ under laboratory air atmosphere. The pyrolysis effluents were detected and quantified with flame ionization detection. The resulting hydrocarbon compounds (HC) thermograms were used to calculate R-index which referred to the thermal stability of SOM. R-index was calculated based on the following equation (Sebag et al. 2016): R =

$\log_{10}((A1 + A2) / A3)$, where A1 (labile biopolymers, 200 to 340 °C) + A2 (resistant biopolymers, 340 to 400 °C) are the integrated area of the HC thermogram below 400 °C and A3 (immature geopolymers) is the integrated area of the HC thermogram between 400 °C and 460 °C. The R-index varies between 0 and 1 and estimates the proportion of thermally stable SOC pool, and the other part (1-R-index) is considered as labile/intermediate SOC pool (Schomburg et al., 2018).

4.2.2.3 Soil aggregate stability

Soil aggregate stability was measured on 5 g of calibrated air-dried aggregates that were sieved through a 3-5 mm sieve. According to the method described by Le Bissonnais (1996), three disruptive tests were used: (1) Fast wetting (FW): rapid immersion in water for 10 min; (2) Wet stirring (WS): slow wetting on -0.3 kPa filter paper for 30 min; and (3) Mechanical breakdown (MB): rapid stirring in 250 mL of deionized water after pre-wetting in ethanol for 30 min. The treated aggregates were collected and transferred to a 50 µm sieve which was immersed in ethanol, and gently moved sieve 5 times by a Hénin apparatus to generate a helicoidal movement. The remained fraction on the sieve were collected, oven-dried at 105 °C and gently dry-sieved on a column of six sieves: 2000, 1000, 500, 200, 100, and 50 µm. The mean weight diameter (MWD) was calculated by the following equation: $MWD = \sum_{i=1}^n X_i \cdot W_i$. Where X_i is the mean diameter of each aggregate size and W_i is the proportion of the total sample weight occurring in the corresponding aggregate size.

4.2.2.4 Enzyme assays

For enzyme analysis, soil samples were collected from the plow layer. In the Qualiagro experiment, 10 soil samples were collected from each plot, while in the PROspective experiment, 5 soil samples were collected from each plot. Fresh soil samples were sieved at a 5 mm sieve and immediately transferred to the laboratory by a cool box and stored at 15°C for backup enzyme analysis. Phosphatase (PHOS), arylsulfatase (ARS), β-glucosidase (GLU), urease (URE), and arylamidase (ARN) activities are measured following ISO 20130 methods (ISO, 2018). Detailed information of enzyme assays was described by Cheviron et al. (2021). Enzymatic activities are calculated and defined as the amount of nanomole equivalent of product released per minute per gram of dry soil (mU g⁻¹ dry soil). Shannon index (Shannon.Enzy) and Simpson index (Simpson.Enzy) of the enzyme were used to describing soil enzyme diversity and calculated as follows: $Shannon.Enzy = -\sum_i^n P_i \times \ln P_i$, $Simpson.Enzy = 1 - \sum_i^n P_i^2$. Where, P_i is the ratio of the relative absorbance value to the total absorbance value for the whole plate.

4.2.2.5 Microbial analysis

Composite soil samples were sieved at 4 mm and sent to the Genosol platform (Dijon, France, www.dijon.inra.fr/plateforme_genosol) lyophilized for further analysis. Total genomic DNA was extracted and purified by using the GenoSol platform named GnS-

GII following the method described by Terrat et al. (2012). The extracted crude DNA ($\mu\text{g DNA/g}$) were used to estimate soil microbial biomass (SMB). The bacterial and fungal communities were analyzed by 454 pyrosequencing of the 16S rRNA and 18S rRNA genes, respectively. Bacterial 16S rRNA gene fragments were amplified using the primers F479 (5' -CAGCMGCYGCNGTAANAC-3') and R888 (5' -CCGYCAATTCMTTTRAGT-3'). The fungal 18S rRNA gene was amplified with primer FR 1 (5' -ANCCATTCAATCGGTANT-3') and FF390 (5' -CGATAACGAACGAGACCT-3'). Finally, the PCR products were purified and pyrosequenced on a GS Roche 454 Sequencing System.

Bioinformatics analyses of generated sequences were carried out using the GnS-Pipe of the Genosol Platform (Terrat et al., 2012). Raw reads were sorted according to multiplex identifier sequences, filtered, dereplicated, and aligned. Sequences with over 95% similarity were considered as a single operational taxonomic unit (OTUs). The kept reads were aligned and classified by taxonomy-based analysis against the Silva database using similarity approaches. In our study, bacterial (Ba) and fungal (Fu) diversity was characterized by OTUs richness, evenness, and Shannon index (Constancias et al., 2015).

4.2.2.6 Nematode community analysis

The composite soil samples were made from 6 individual subsamples taken with an auger from the plow layer of each plot. Collected samples were immediately sealed in a bag and sent to the ELISOL environment laboratory (ELISOL Laboratory, Montpellier, France). Soil nematodes were extracted from 100 g fresh soil using a modified centrifugal flotation method (Jenkins, 1964) followed by cotton-wool filter method (ISO 23611-4), and were counted at low magnification under a binocular microscope (magnification 40 \times). The composition of the soil nematode community was determined by inverted compound microscope after fixation in 4% formalin solution and transfer to mass slides. The nematodes were assigned to the following trophic groups characterized by feeding habits (1) bacterivores (BFN); (2) fungivores (FFN); (3) omnivore-carnivores (OPN), and (4) plant feeders (PFN) following Yeates et al. (1993).

Enrichment (EI) and Structure (SI) were calculated as $EI = 100 \cdot e / (e + b)$, $SI = 100 \cdot s / (s + b)$, where e , s , and b are enrichment, structure, and basal food web components. Nematode channel ratio (NCR) was calculated as $NCR = BFN / (BFN + FFN)$, where BFN and FFN are abundances of the nematode fauna allocated to bacterivorous and fungivorous groups (Yeates, 2003); Maturity index (MI) and plant parasite index (PPI) were calculated as $MI = \sum [v(i) \times f(i)]$, $PPI = \sum [v'(i) \times f'(i)]$, where $v(i)$ is colonizer-persister (c-p) value of taxon and $f(i)$ is the frequency of taxon i in a sample (Bongers, 1990; Yeates, 1994). Shannon index of nematodes (H' Nematode) were calculated as: $H' \text{ Nematode} = -\sum_i^n P_i \times \ln P_i$, where P_i is the proportion of genera n_i in the total nematode community n .

4.2.2.7 Cumulative flux balance

To evaluate the effects of flux balance on soil properties, the balance of C, N, P, K, and TE were calculated as follows:

$$C_{balance} = C_{applied} + C_{crop_residue} \quad (Equation\ 4-1)$$

$$N_{balance} = N_{applied} - N_{export} \quad (Equation\ 4-2)$$

$$P_{balance} = P_{applied} - P_{export} \quad (Equation\ 4-3)$$

$$K_{balance} = K_{applied} - K_{export} \quad (Equation\ 4-4)$$

$$TE_{balance} = TE_{applied} - TE_{export} \quad (Equation\ 4-5)$$

where $C_{applied}$, $N_{applied}$, $P_{applied}$, $K_{applied}$, and $TE_{applied}$ are the cumulative sum of C, N, P, K, and TE input (as OW, mineral fertilizer, and digestate), respectively; $C_{crop_residue}$ is the cumulative C return by crop residues (maize for QUA, all the crops in COL); N_{export} , P_{export} , K_{export} and TE_{export} are the cumulative sums of uptake in harvested parts of the crops (also wheat residues in QUA). The detailed input and export of each OW treatment and control are presented in Table S6 to Table S9.

Then, the $\Delta C_{balance}$, $\Delta N_{balance}$, $\Delta P_{balance}$, $\Delta K_{balance}$, and $\Delta TE_{balance}$ were calculated as the difference between the respective balance values of each OW treatment and their corresponding control.

4.2.3 Soil quality index

Soil quality index (SQI) is an effective tool for assessing the effect of soil management and enhances the understanding of soil ecosystems (Raiesi, 2017). Because of its flexibility and ease of use, SQI have been successfully used at many scales and locations. For example, Obriot et al. (2016) use this approach to evaluate the effects of OW applications on soil and crop quality in QualiAgro. However, limited studies have used SQI to investigate performance of different OW across sites. Therefore, evaluating soil quality status under different OW applications is rather critical for developing sustainable field management.

The first step in this method is the selection of representative soil indicators which are sensitive to the environment. We performed a standardized principal-components analysis (PCA) to reduce the dimension of the selected indicators (Andrews and Carroll, 2001). Principal components (PCs) with eigenvalues greater than one (Brejda et al., 2000) and which explained at least 5% of the variation in the data (Wander and Bollero, 1999) were selected for the minimum data set (MDS). When more than one indicator was retained within a single PC, multivariate correlation was used to determine if they were redundant. Highly correlated indicators ($r > 0.70$) were considered redundant and only the indicators with the highest loading was retained in the MDS (Andrews et al., 2002).

During the analysis, the ability to interpret the uncorrelated components was enhanced by performing a varimax rotation (Flury and Riedwyl, 1988).

After determining the variables for the MDS, each observation of each MDS indicator was ranked in ascending (more is better) and descending (less is better) order. For each type of indicator, the following equation was used:

$$y_{ascending} = \frac{x-s}{t-s} \quad (\text{Equation 4-6})$$

$$y_{descending} = 1 - \frac{x-s}{t-s} \quad (\text{Equation 4-7})$$

where x values of soil property converted into a 0–1 scale, s is the lowest soil property value and t is the highest soil property value.

After the transformation, the MDS variables for each observation were weighed using the PCA results. The weight for the MDS was calculated as follow:

$$W_i = C_i / \sum_{i=1}^n C_i \quad (\text{Equation 4-8})$$

where W_i is the weighting of the indicators, C_i is the communality value of the indicator, and n is the number of indicators included in the MDS. Finally, the Soil Quality Index equation (SQI) was calculated as follows (Doran and Parkin, 1994):

$$SQI = \sum_{i=1}^n S_i W_i \quad (\text{Equation 4-9})$$

where n is the number of variables integrated in the index; W_i is the weighting factor; S_i is the indicator score for each variable. Higher index scores indicate better soil quality.

4.2.4 Statistical analysis

To test the effect of OW on soil physical, chemical, and biological characteristics, one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$ level of significance) was used for separation of the means within each experiment. The Pearson correlation coefficient was used to examine relationships among measured variable. In addition, simple linear regression was used to detect the relationship between the $\Delta TE_{balance}$ and ΔTE_{stock} .

Gradient boosting model (GBM) proposed by Friedman (2001) was performed to reveal the relative influence of OW input in the regulation of soil physical, chemical, and biological characteristics. To avoid the heterogeneity of sites, the $\Delta C_{balance}$, $\Delta N_{balance}$, $\Delta P_{balance}$, $\Delta K_{balance}$, which were mentioned in 4.2.2.7 were set as predictor variables and the difference of physical, chemical and biological characteristics of each OW treatments with their corresponding control were set as the target in GBM. GBM, intended for robustness, is principally an ensemble model made of multiple executions of another model called Classification and Regression Tress (CART). In GBM modeling, four parameters require to be specified: interaction.depth (the maximum number of

nodes per tree), n.trees (the number of gradient boosting iterations), shrinkage (the learning rate), and n.minobsinnode (the minimum number of observations in the tree's terminal nodes). Here, in order to optimize the hyper-parameters for the algorithms, we used a grid search and looked at the following hyper-parameters: the interaction.depth was set from 1 to 10 with a single interval. The n.trees was set in the range of 100 to 1000 with an interval of 100, whereas the n.minobsinnode was tested from 2 to 10 with an interval of 2. Three-degree levels included 0.01, 0.05, 0.1 were tested to find the optimized shrinkage level. Then, we chose the best GBM model with the smallest root mean square deviation (RMSE). We evaluated the performance of the calibration results using a 5-fold cross-validation (CV) method. The entire dataset was randomly partitioned into 5 folds, where 4 folds of data were used to build the ensemble-based model, while the remaining folds were used for model validation. The GBM model was implemented using the 'gbm' package in R 4.0.4 (R Core and Team, 2019).

4.3 RESULTS

4.3.1 Control treatment at the 2 sites

There were significant differences between the control treatment in the final year (2018) of COL and QUA. COL was characterized by higher soil C, N, and K content and also with higher soil pH and CEC (Table S10). For the biological characteristics, limited differences occurred. COL has much higher bacterial feeder nematodes and fungal-feeder nematodes whereas QUA has much higher arylsulfatase and arylamidase activities. However, TE availability showed significant differences at the 2 sites. QUA was rich in extractable Cd and Ni whereas COL had higher Cu and Pb availabilities.

Limited differences occurred between the two control treatments within each site. For COL, COL_N- has larger MWD.FW and MWD.MB compared with COL_N+. Phosphatase and β -glucosidase activities were higher in COL_N+. Fungal-feeder nematodes in COL_N+ were higher than in COL_N- while NCR was lower in COL_N+. In QUA, QUA_N has larger soil C and soil pH whereas QUA_LEG has higher MWD.FW. Plant-feeder nematodes and PPI in QUA_LEG were significantly higher than in QUA_N.

4.3.2 General effects of OW

Long-term OW fertilization markedly changed the soil physical, chemical and biological characteristics at both sites compared with the respective control treatments (Figure 4-1).

OW application significantly improved soil chemical properties. TC, TN, AP, and EK significantly increased in most OW application treatments except in the SLU_N- in COL in which EK slightly decreased. Soil pH changed according to the sites: it slightly decreased in COL while it increased in QUA for most of OW treatments. Soil CEC increased in all the OW treatments of QUA and GWS_N-, SLU_N+, GWS_N+, and

BIO_N+ in COL. Based on the Rock-Eval thermal analysis, both Active C and Stable C in soil increased in most treatments of QUA. However, Active C increased more than stable C, so the proportion of Stable C decreased. In COL, GWS_N-, GWS_N+, BIO_N+ and FYMC_N+ increased Stable C. For most treatments in QUA, extractable soil trace element contents significantly increased after OW application (except for Ni). In contrast in COL, mainly Zn and Ni increased, while Pb and Cu increased only in BIO_N+ and SLU_N+, respectively.

The aggregate stability estimated with MWD.MB did not show a significant increase in any treatment at both sites and even decreased significantly in the FYMC_N+ treatment in COL. MWD.FW increased only in the FYMC_N+ and BIO_N+ in COL, with no significant change compared to the control observed in QUA. The changes in MWD.WS was most pronounced among the three methods, with increases observed in the BIO_N, GWS_N, and FYM_N in QUA, as well as in the FYM_N- in COL.

The effects of OW application on soil enzyme activities varied among the different types of OW and study sites, with more pronounced effects observed in QUA compared to COL. In site QUA, all OW applications significantly increased β -glucosidase, urease, and arylamidase activities, while only the GWS application increased phosphatase. The effects were similar whether OW application were stopped or not in 2014, and whether alfalfa was cropped or not. In site COL, treatments without mineral N (COL_N-) showed stronger changes on enzyme compared with mineral N treatments (COL_N+). SLU_N- significantly increased β -glucosidase, arylsulfatase, and arylamidase activities, BIO_N- significantly increased β -glucosidase and arylsulfatase activities, FYM_N- significantly increased arylsulfatase and urease activities and FYMC_N- only increased arylsulfatase activities. GWS_N-, SLU_N+, GWS_N+, BIO_N+, and FYMC_N+ had no significant effects on any kind of enzyme. BIO application had the opposite effect on the Shannon index of enzyme activities at the two sites: BIO decreased Shannon index of enzyme in site COL, whereas it increased in site QUA.

Soil microbial biomass significantly increased in all the OW treatments in QUA as well as the OW treatments in COL_N-, whereas it kept relatively stable in OW treatments in COL_N+. The different index describing the diversity and composition of bacterial and fungal communities (richness, evenness, and Shannon index of bacterial and fungal) did not change significantly with the type of OW applied, except the Richness of fungal biomass that slightly decreased in FYM_N- in COL.

The effect of OW application on soil nematode communities was not as dramatic as that of enzymes, especially in COL. The abundance of BFN was significantly enhanced by the application of BIO_N and FYM_N in QUA, while it remained stable in the other treatments. FFN increased by MSW application in QUA_N whereas it decreased by SLU application in COL_N-. OPN was dramatically decreased by OW application in QUA_LEG and insignificantly dropped in QUA_N. Conversely, an increase was noted in the FYM_N treatment in QUA_N. PFN increased in FYM_N+ and FYMC_N+ in COL as well as

GWS_LEG in QUA. The community indices of nematodes showed that EI, SI, NCR, MI, and Shannon index of nematodes remained stable in COL. Only PPI decreased in GWS_N- and FYM_N-. However, in QUA, EI, NCR, PPI, and Shannon index of nematodes increased in some OW treatments whereas SI and MI decreased.

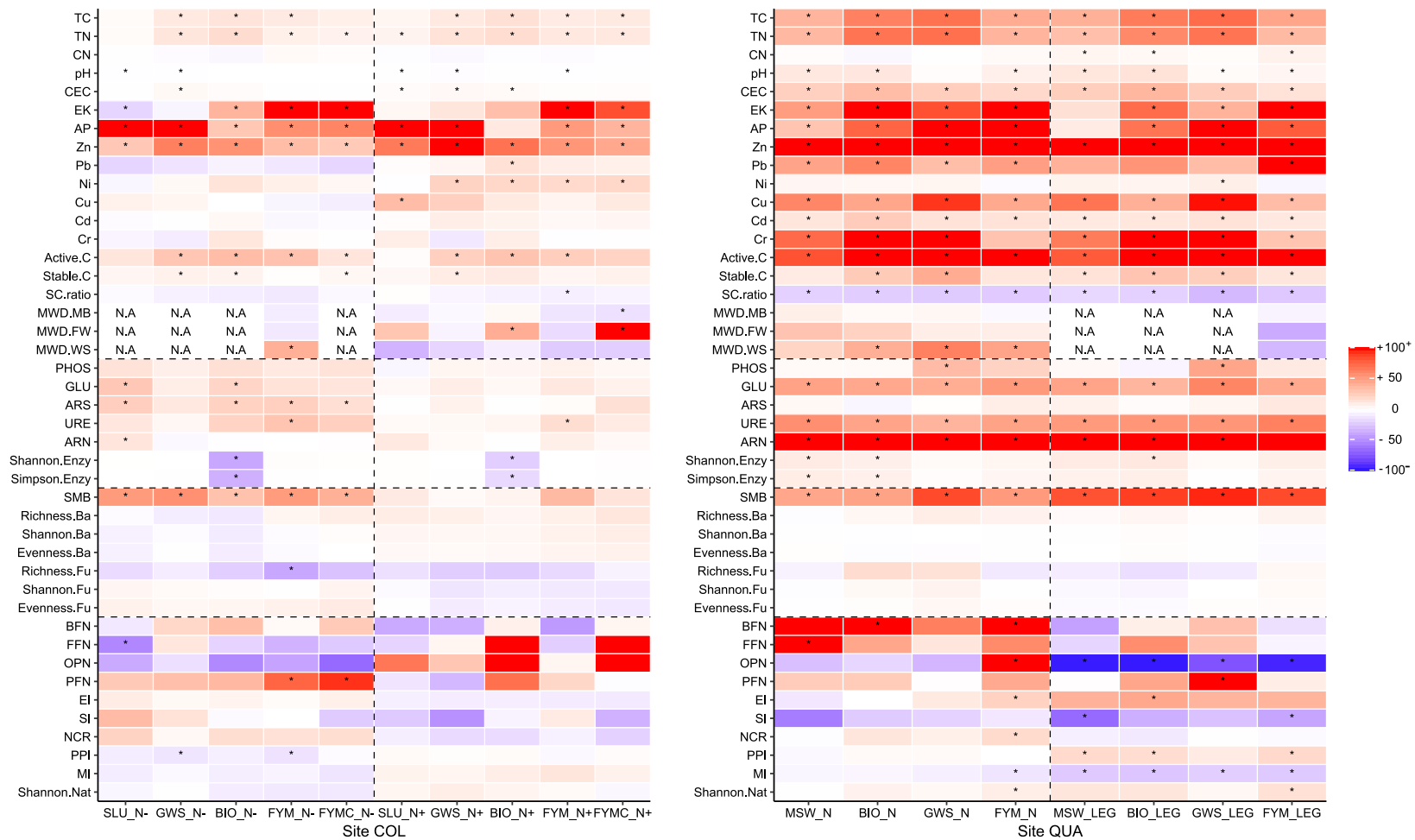


Figure 4-1 Overview of the effects of OW application on soil enzyme, microbe, nematode, and physical and chemical properties in QUA and COL. The color indicates the proportion of change compared to the control treatment. The darker the color, the stronger the proportion of change (red: increase; blue: decrease). The data from each experiment (COL_N-, COL_N+, QUA_N-, QUA_LEG) are used separately for statistics. The * indicates a significant difference with the control treatment without OW ($P < 0.05$). The mark N.A indicates the data were not measured. The value of the plots are shown in Table S11 for COL and Table S12 for QUA. For the abbreviations used in this figure, please refer to the List of Abbreviations section.

Overall, the results indicated that significant changes were mostly observed in the physico-chemical properties of the soil (Figure 4-2), while limited changes were observed in soil biological characteristics and aggregate stability. Furthermore, a greater number of changes were observed in QUA than in COL.

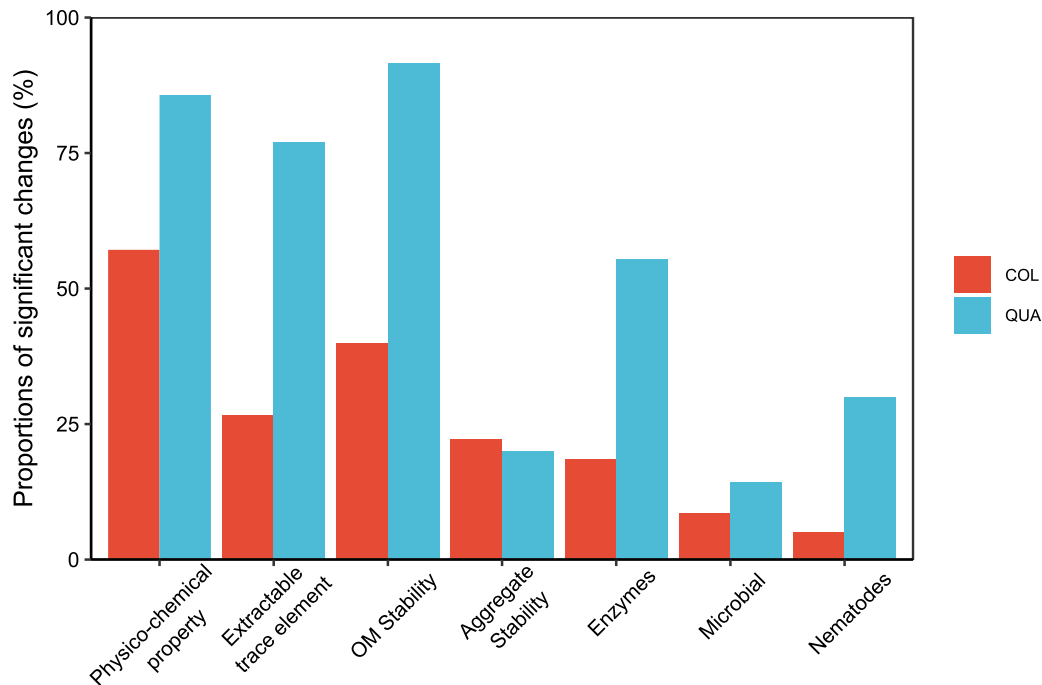


Figure 4-2 Proportions of significant changes between the OW treatments and their respective control treatments per group of variables (all OW treatments and variables together) for the COL and QUA sites.

4.3.3 Correlation analysis

The correlation analysis of the measured indicators was performed and the results are shown in Figure 4-3. Globally, almost all variables were correlated together (negatively or positively), except the diversity index such as Shannon and Simpson's index of enzyme activities, Shannon index of nematode and variables related to microbial biomass diversity. TN and TC were related to most of the soil biological index. Positive correlations were observed between β -glucosidase, urease, arylamidase, soil microbial biomass, BFN, PFN, EI, and PPI with TC, while negative correlations were found for OPN, SI and MI with both TN and TC. There were positive correlations between extractable TE and enzymes except for arylamidase. On the contrary, a negative correlation was observed between most of the extractable TE and nematodes, including BFN, FFN, OPN, and PFN, depending on the type of trace element. pH and CEC were also correlated with many other variables. However, the correlation depended on the type of variables. For example, β -glucosidase and arylamidase were positively correlated with pH and CEC whereas phosphatase, arylsulfatase, and urease were negatively correlated with pH and CEC.

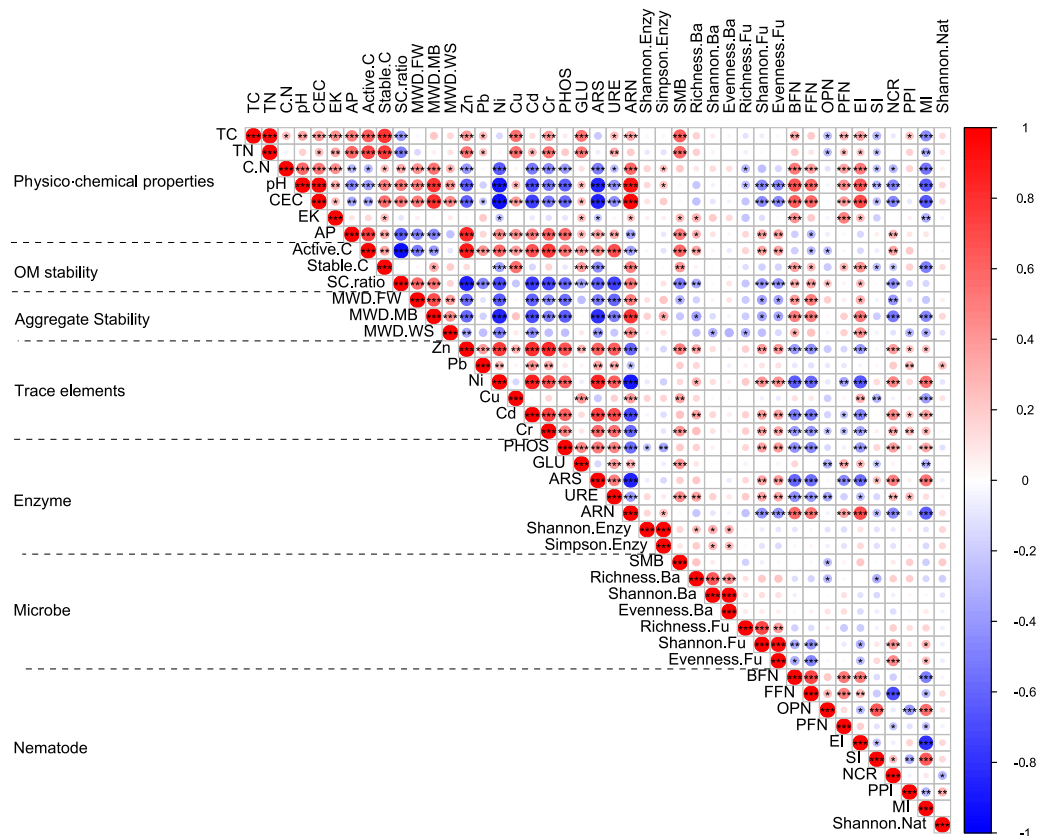


Figure 4-3 Correlation matrix of soil physical, chemical and biological properties. (Red colors represent positive correlations, while blue colors denote negative correlations. The saturation of color represents the level of correlation, the darker the color, the stronger the correlation. The statistical were based on the raw data of QUA and COL. P values of each correlation coefficient are shown in the figure: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). For the abbreviations used in this figure, please refer to the List of Abbreviations section.

4.3.4 Soil Quality Assessment

4.3.4.1 Minimum data set

The results of the PCA method showed that six principal components (PCs) had an eigenvalue greater than one and the cumulated explained variance was 74.8% (Table 4-1). In PC1, CEC, pH, arylsulfatase, arylamidase, Cd, and Ni had the highest loading value but these indicators were markedly correlated, thus we only kept the Ni, which had the highest loading value of the soil properties to reflect PC1. For PC2, TC and TN shown the highest loading values. They were well correlated and TC were therefore excluded from the MDS. Nematode indicator OPN and soil indicator EK were in relation to PC3 and PC4, respectively. For PC5, we selected the Shannon index and Simpson's diversity index of enzymes, and after examining the correlation results, only the Shannon index was included in the MDS. The Shannon index of nematode with the highest loading value was chosen in PC6. In summary, only TN, EK, Ni, Shannon index of enzyme, OPN, and Shannon index of nematode were finally selected into the MDS, which were further weighted and scored to calculate the soil quality index (SQI).

Table 4-1 Principal components, eigenvalues and component matrix variables. The analysis is based on the raw values of all treatments at both sites. Only the indicators with the higher loading values are shown. Name of abbreviations can be found in the abbreviation table.

Component	PC1	PC2	PC3	PC4	PC5	PC6	Communality	Weight
Eigenvalue	10.4	6.0	2.9	2.8	2.3	1.8		
Proportion of variance %	29.8	17.1	8.2	8.1	6.6	5.1		
Cumulative variance %	29.8	46.9	55.1	63.2	69.7	74.8		
Factor loadings								
TC		0.87	0.20	0.29		0.19	0.93	
<u>TN</u>		0.92	0.12	0.15	-0.12	0.13	0.91	0.18
pH	-0.86	0.16	0.19	0.30	0.11		0.91	
CEC	-0.90	0.27	0.14	0.21			0.95	
<u>EK</u>		0.18		0.83			0.73	0.17
Cd	0.87	0.22					0.82	
<u>Ni</u>	0.94			-0.24			0.94	0.19
ARS	0.88	-0.10	-0.11	-0.17			0.83	
ARN	-0.88	0.24	0.16	0.20	0.16		0.92	
<u>Shannon.Enzy</u>					0.95		0.91	0.18
Simpson.Enzy	-0.14				0.95		0.93	
<u>OPN</u>	-0.14	-0.17	-0.87			0.13	0.84	0.17
<u>Shannon.Nat</u>		0.13			0.20	0.66	0.52	0.10

Note: Bold font values are considered highly weighted. Underlined indicators loadings selected as MDS. Complete table is shown in Table S13.

4.3.4.2 Soil quality indices under different OW application

Based on the selection of the MDS, we evaluated the SQI at the two sites (Figure 4-4). We found that the SQI was lower in both control treatments of QUA as compared to COL. Moreover, CON_N- was statistically higher than CON_N+ in COL whereas there was no statistical difference between CON_N and CON_LEG in QUA. However, the application of OW in QUA resulted in a higher increase in SQI as compared to COL. In QUA, all OW applications significantly increased SQI compared with the control ($p < 0.05$), except for MSW_LEG. In COL, only FYM increased SQI significantly in both parts (COL_N- and COL_N+). It's interesting to see BIO_N- had lower SQI compared with the control (COL_N-).

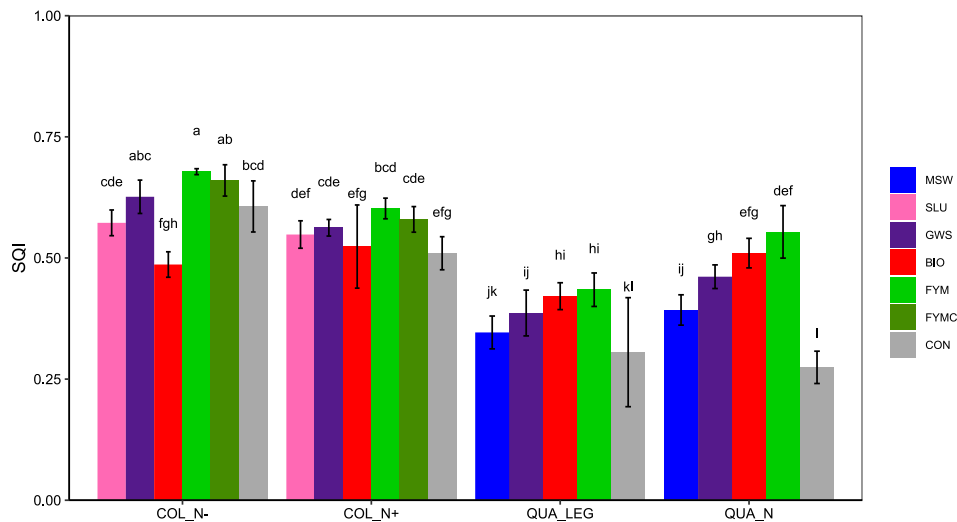


Figure 4-4 Mean comparisons of SQI in (a) site COL and (b) site QUA. The analysis is based on the raw values of all treatments at both sites. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$. Different letters indicate statistically significant differences at a P -value of 0.05 (Duncan test). Error bars are the standard deviations (four replicates). For the abbreviations used in this figure, please refer to the List of Abbreviations section.

4.3.5 Effects of OW balance on soil property changes

4.3.5.1 Nutrient driven soil properties change

The predicting performance of the GBM model can be observed in Figure 4-5. The models constructed by GBM can reasonably predict the soil physico-chemical properties. Except for soil C/N ratio, the R^2 of the 5-fold CV of other indicators has achieved satisfactory results (with $CV-R^2 > 0.6$). The performance of the model in predicting soil enzyme activities varied widely across different indicators. Specifically, the model showed high accuracy in predicting urease, Shannon index of enzyme, and Simpson index of enzyme (with $CV-R^2 > 0.7$), but performed poorly in predicting arylsulfatase (with a $CV-R^2$ of 0.21). However, the model has shown an overall poor performance on soil microbial indicators as well as nematode indicators.

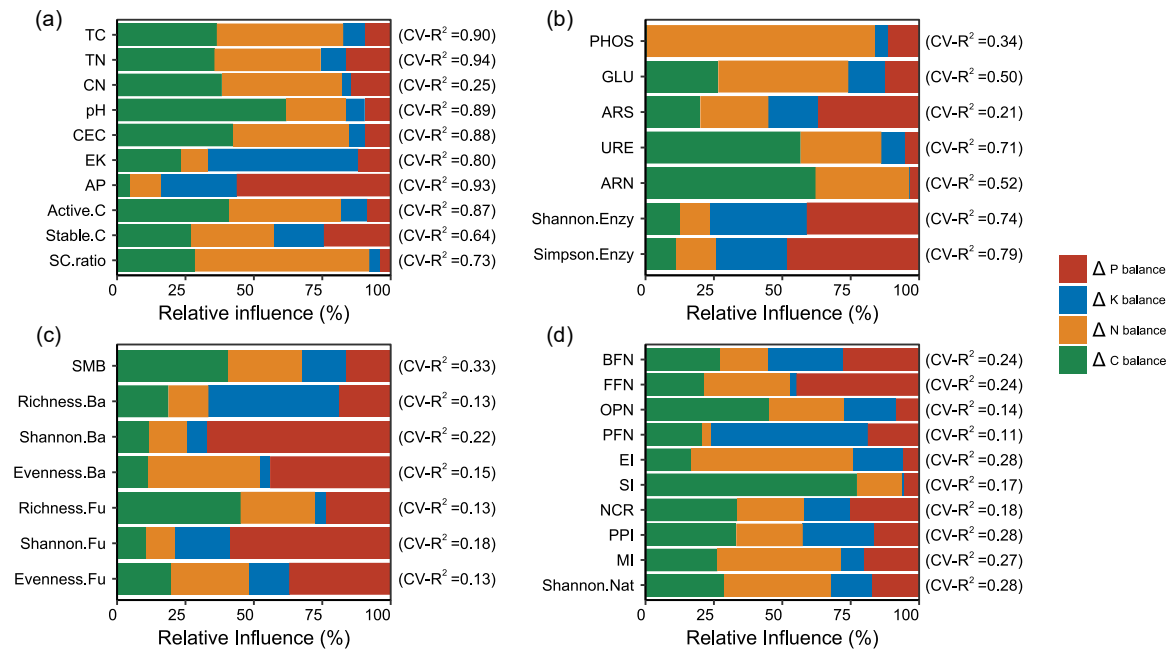


Figure 4-5 The 5-fold cross-validated R^2 (CV- R^2) of GBM and the relative contributions (%) of predictive variables (total P, K, N, and C balances) for the GBM model of (a) soil physico-chemical characteristics (b) soil enzyme activities (c) soil microbial and (d) soil nematodes. The length of the predictive variables represents the degree of their contribution to the overall prediction. For the abbreviations used in this figure, please refer to the List of Abbreviations section.

The relative contribution of each considered flux of elements is shown in Figure 4-5. The C and N balance played a critical role in predicting the changes in soil physico-chemical properties. For example, they accounted together for 74% of the influence on soil pH and CEC. Soil AP and EK were mainly determined by the P and K balance, respectively. For enzyme activities, C balance was the primary factor affecting urease and arylamidase, while N balance was the major factor affecting phosphatase and β -glucosidase. P and K balance was the main explanatory factor for Shannon and Simpson's diversity index of enzyme activities.

4.3.5.2 Soil available trace elements changes

The EDTA-TE stocks in soil increased with cumulative TE balance. Our regression slopes showed that a 100 g/ha of Δ cumulative Zn balance, Δ cumulative Pb balance, Δ cumulative Cu balance, Δ cumulative Cd balance, and Δ cumulative Cr balance increased of 69, 89, 52, 58, and 4 g/ha Δ EDTA-Zn, Δ EDTA-Pb, Δ EDTA-Cu, Δ EDTA-Cd and Δ EDTA-Cr stocks, respectively (Table 4-2). However, for Ni, no linear relationship was found between the Δ Ni balance and Δ EDTA-Ni stocks.

Table 4-2 Δ Cumulative TE balance and associated variation in Δ EDTA-TE stock in the soil plowed layer of all the treatments of QUA and COL considered together

Treatments	Δ EDTA-TE stock vs Δ Cumulative TE balance regression			
	Intercept	Slope	P-value	R ²
Zn	-7077 (2151)	0.69 (0.06)	< 0.001	0.88
Pb	-2496 (1849)	0.89 (0.19)	< 0.001	0.59
Ni	-98 (65)	0.04 (0.03)	0.15	0.12
Cu	-986 (965)	0.52 (0.08)	< 0.001	0.75
Cd	-10 (9)	0.58 (0.09)	< 0.001	0.56
Cr	-33 (51)	0.04 (0.01)	< 0.001	0.53

To investigate the differences in TE dynamic between the two sites, we determined the relationship between Δ EDTA-TE stocks in the plowed layer vs. Δ cumulative TE balance for COL and QUA, respectively (Figure 4-6). For Zn, a steeper regression slope was found in QUA, which means the same Δ Zn balance will increase larger Δ EDTA-Zn content in QUA compared to COL. For Ni and Cu, a positive relationship was found between Δ EDTA-Ni stocks and Δ Ni balance and Δ EDTA-Cu stocks and Δ Cu balance in QUA, whereas no statistically significant relationship was observed in COL.

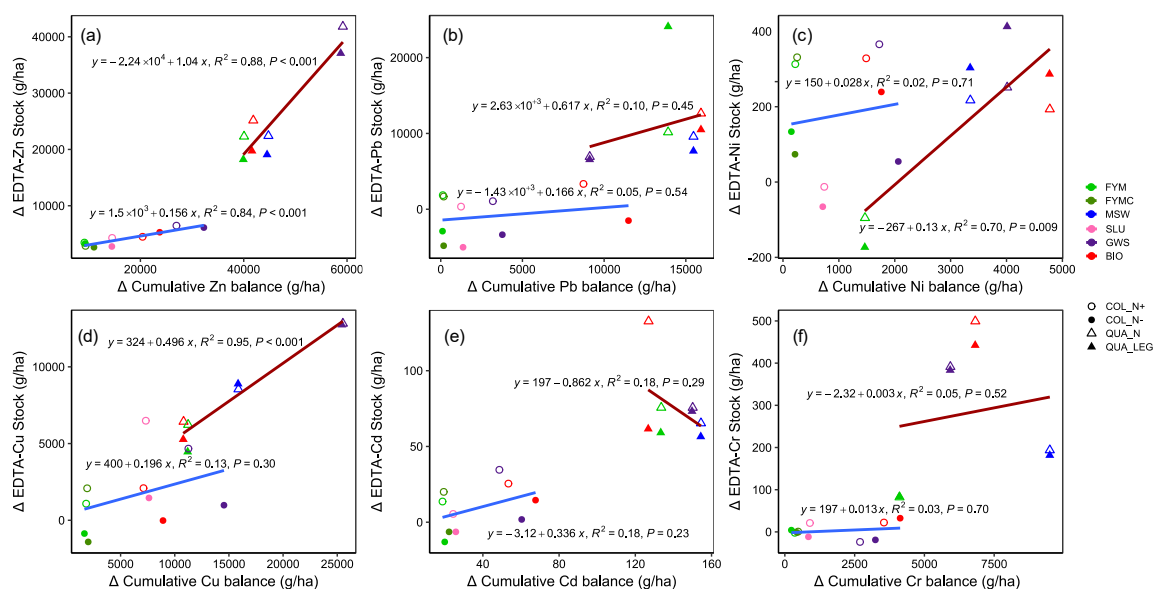


Figure 4-6 Relationship between (a) Δ cumulative Zn balance and Δ EDTA-Zn stock, (b) Δ cumulative Pb balance and Δ EDTA-Pb stock, (c) Δ cumulative Ni balance and Δ EDTA-Ni stock, (d) Δ cumulative Cu balance and Δ EDTA-Cu stock, (e) Δ cumulative Cd balance and Δ EDTA-Cd stock, (f) Δ cumulative Cr balance and Δ EDTA-Cr stock in the soil plowed layer for COL and QUA. For the abbreviations used in this figure, please refer to the List of Abbreviations section.

4.4 DISCUSSION

4.4.1 General effect of OW application on soil properties and main interactions

Repeated OW application increased soil C content at both QUA and COL sites, which is in line with numerous studies (Gross and Glaser, 2021; Maillard and Angers, 2014). It is important to note that the increase in carbon input resulting from the greater amount of crop residues due to OW application cannot be ignored as a contributing factor to SOC increased. Both soil active C and stable C increased after OW application at both sites (at least for certain OW), whereas the ratio of stable C decreased, suggesting that C inputs mainly accumulated in labile soil C pools. It is well known that OW markedly increase soil active C by directly contributing with labile C and indirectly by enhancing microbial activities that convert plant residue-C into active C (Li et al., 2018; Poirier et al., 2013). Total and active C were indeed correlated with many soil biological variables in our study (Figure 4-3). The increase in stable C could be related to the biochemical resistance of organic-C compounds contained in OW (Ding et al., 2012). Complex organic molecules such as lignin and lignin-like products are difficult to decompose and therefore contribute to the resistant C pool (McLauchlan et al., 2006), even if the effect of biochemical resistance in the long term is questioned (e.g., Dignac et al., 2017).

The total amounts of nutrients applied within OW, directly increased soil TN, AP, and EK (Figure 4-5) at the two study sites. In addition to OW nutrient inputs, Ohno et al. (2005) suggested that increasing the concentration of soil dissolved organic C (DOC) may increase the bioavailability of soil P. Although we did not measure DOC in our study, we observed a significant correlation between total C and available P at the two sites (Figure 4-3).

Previous studies have shown that OW application had a positive effect on the stability of aggregates (Chen et al., 2021; He et al., 2018; Zhang et al., 2014). In our study, we found that the OW application increased only MWD.WS and MWD.FW in some of the OW treatments in QUA and COL. The increased in MWD could be attributed to the increased microbial activity that induces binding of soil particles into macroaggregates (Six et al., 2004). Despite the observed improvements in SMB, we did not find a positive relationship between MWD and SMB in our study. Besides, OW application could stimulate plant root growth therefore bind fine soil particles and form stable aggregates (Chen et al., 2021). However, the increase in aggregate stability in our study was limited compared to several previous studies (Chen et al., 2021; He et al., 2018; Zhang et al., 2014), especially the study of Annabi et al. (2011) also conducted in QUA. This highlights that the temporal variability of aggregate stability extends beyond its relationship with SOC.

The incorporation of OW to soil influences soil enzymatic activities because the OW supply readily metabolizable C (Tejada et al., 2006). Chang et al. (2007) reported that the urease, β -glucosidase, and arylsulfatase activities were significantly linearly

correlated with soil organic matter contents. We also observed this significant correlation at our two sites for urease, β -glucosidase, and arylsulfatase. The influence of soil pH on soil enzyme activities was also found in our study. Soil pH changes soil enzyme activities by altering ionization and solubility of the soil enzyme substrates and enzyme factors (Tabatabai, 1994). In addition, OW may contain intra- and extracellular enzymes that can stimulate enzymatic activities in the soil (Pascual et al., 1998). It was reported that enzymatic activity might be diminished with increasing concentration and availability of TE (Moreno et al., 2003). However, our results indicated that TE did not negatively affect phosphatase, β -glucosidase, arylsulfatase, and urease activity, only arylamidase activities were negative correlated with TE availability (Figure 4-3). It might be because the negative effects of TE strongly depend on their amounts and could be masked by the positive effect of OW application (Albiach et al., 2000).

OW application resulted in a microbial biomass increase but have limited influence on the diversity of bacteria and fungi in both QUA and COL. The C and N balance are the major factors driving the soil microbial community changes (Figure 4-5), which is consistent with the findings of Cederlund et al. (2014). OW addition promotes microbe growth and reproduction by introducing potential substrates. Additionally, the microbe naturally existing in OW could also enhance soil microbial biomass (Dong et al., 2014). Previous studies have highlighted the importance of pH on regulating microbial biomass, because microbes need more energy consumption to overcome acidic stress and maintain cellular integrity (Jones et al., 2019). However, because of the neutral to high pH at the two sites, we didn't observe the relationship between SMB and soil pH in our study. OW application did not change soil bacterial diversity and we only found that FYM application decreased the fungi richness. In a four years experiment carried on calcareous soil, Bei et al. (2018) reported that OW application did not lead to significant soil bacterial or fungal diversity change. However, the decrease of the fungi richness with FYM application is in line with Sun et al. (2020). The author believed it is because dung and other saprotroph introduced by FYM compress the living niche for other fungi taxa and lead to the extinction of the other taxa which had a low competitive advantage.

Repeated application of OW led to a significant increase in TE availability, in line with numerous previous studies (Benke et al., 2008; Hussain et al., 2021). Because of the TE contents in OW and high application rates, the TE inputs were much higher than the plants removal and thus accumulated in soils (Table S8 and S9). On the other hand, OW application to soils could release soluble organic complexes, therefore influencing the mobility and availability of TE (Martínez et al., 2020). It is known that humic substances, the products of OW humification, containing carboxyl, hydroxyl, and phenolic functional groups easily complex with metal ions (Laborda et al., 2008). In our study, we found that SOC was positively related to the availability of Zn, Pb, Cu, and Cr (Figure 4-3); the concomitant increase of TE input with the increase of C input (leading to a higher SOC) having a priori more impact on available trace elements.

4.4.2 Differences between the OW and sites on soil properties change

We found a more pronounced change in soil physical, chemical, and biological characteristics in QUA compared with COL (Figure 4-2). This was mainly due to the higher OW application rates in QUA. OW application brings exogenous C, N, P, and K input and therefore results in soil properties change (Figure 4-5). Because all cereal crop residues were exported in QUA, the effects of OW were also strengthened as OW represented relatively more C input in comparison to COL where crop residues were returned to soil. In addition to the higher application rates discussed above, the different native soil properties between the two sites also result in the different responses of soil properties change after OW inputs. Compared with COL, QUA was characterized by a lower SOM content (Table S10). A study by Yanardağ et al. (2017) demonstrated that the native SOM determined the response of microbial communities to OW input and soils with lower SOM led to the largest enzyme activities and microbial biomass increase. Moreover, COL was rich in calcium carbonate leading to an alkaline pH which could also influence microbial communities strongly and therefore result in the different biological responses (Lejon et al., 2007). For example, we have observed that OW inputs increased arylsulfatase activities in COL whereas it did not happen in QUA, probably because Ca play an important role in arylsulfatase activity (Hao Chen et al., 2019). Moreover, soil texture also plays an important role in enzyme activities. Even when the same OW was applied to soils with different textures, the activity and kinetics of arylsulfatase varied (Khadem et al., 2019). In addition, we found that the relationship between soil TE and the TE balance varies between the two study sites. This variability may be due to differences in climatic conditions (Punia, 2021), soil characteristics (Wenzel et al., 1992), and planted crops (Padmavathamma and Li, 2012) among the sites, which could affect TE dynamics. It was reported that soil carbonates may also control retention and sorption of TE in calcareous soils (Jalali and Jalili, 2011).

The change in soil physical, chemical, and biological properties also depends on the type of OW. GWS that was rich in P resulted in the highest increase in soil AP at both sites. GWS also stimulated the activity of phosphatase in QUA, contrary to the other OW. It has been previously reported that the activity of phosphatase is enhanced by N fertilization, regardless of biome, community, or climatic conditions (Margalef et al., 2021). In our study, the GBM model also highlighted the importance of N balance on phosphatase activity (Figure 4-5), and GWS had the highest N balance among the OW used (Table S7), which may have contributed to the observed increase in phosphatase activity. On the other hand, GWS had a higher concentration of Zn and Cu balance and therefore increased the highest TE content in soil (Table S8 and S9). It was reported that SLU had a lower humification coefficient and was easily degradable (Chen et al., 2022). Thus, SLU failed to increase SOC. FYM and FYMC were characterized by the highest K content classically observed in dairy manure. Therefore, they led to the highest EK content in soil. In addition, it was observed that only FYM treatment increased urease activity in COL, which could be explained by its higher C balance. Our GBM model showed a good fit for urease activity and indicated that C balance is the

major factor driving the increase in urease activity (Figure 4-5), which is consistent with the findings of Zantua et al. (1977), who suggested that organic matter content was the main contributor to variation in urease activity. Both FYM and FYMC increased the plant-feeder nematodes in COL, whereas FYM decreased PPI. These results were in line with the study by Nahar et al. (2006) who suggested that raw manure could be more effective compared with composted manure in reducing plant parasitic nematodes.

According to Richard (1992), composting could concentrate heavy metals due to the loss of carbon and water by microbial respiration. In our study, the total element (TE) content in FYMC and GWS was not significantly higher than in FYM and SLU, respectively (Table S3). However, due to differences in fertilization strategy, FYMC and GWS resulted in higher inputs of TE compared to FYM and SLU, respectively (Table S8). Despite this, there were no significant differences in the trace element content of the soil between these treatments (Figure 4-1 and Table S10). MSW was characterized by a lower P content and a higher C mineralization rate (Levavasseur et al., 2022). Thus, MSW increased the lowest TC and failed to increase AP in soil. Moreover, the high C/P rate in the soil after MSW application could directly affect the microbes community, therefore increasing enzyme diversity (Zhang et al., 2022). Interestingly, we found that only BIO significantly decreased Shannon and Simpson index of enzymes in site COL. We speculate that the heterogeneity of BIO used in COL and QUA, which were made from locally collected biowaste compost, may have contributed to the observed differences in enzyme diversity changes. Different substrate induce the synthesis of different enzyme by soil microorganisms, leading to varying effects on enzyme diversity.

4.5 CONCLUSIONS

Our results confirmed that the long-term OW application significantly changes the soil physical, chemical, and biological properties. The effects highly depended on the application rate, OW types, and soil native condition. The effects of OW on improving soil properties may be limited in soils with already high fertility like in COL. Incorporation of crop residues into the soil may reduce the noted impact in COL. Moreover, biological properties and aggregate stability were less affected compared with soil chemical properties. Regular application of OW may be insufficient to improve aggregate stability, but it can increase soil microbe biomass and enzyme activities without adversely affecting soil biological activity, which is a common concern. Thus, OW amendments incorporated with other practices (i.e., reduced tillage, cover crops, rotation diversity) could be a judicious strategy for improving soil structure and biological properties. It is worth noticing that OW improve soil properties accompanied by an increase in TE availability. Therefore, it is advisable to be aware of the potentially detrimental effects of TE when recycling OW in the long term. Future studies are also strongly recommended moving towards a combination of two or more management strategies in field experiments to improve all components of soil fertility.

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Chapter V

An 18-year field experiment to assess how various types of organic waste used at European regulatory rates sustain crop yields and C, N, P, and K dynamics in a French calcareous soil

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The only difference between this chapter and the published paper is the numbered references to figures and tables.

5 AN 18-YEAR FIELD EXPERIMENT TO ASSESS HOW VARIOUS TYPES OF ORGANIC WASTE USED AT EUROPEAN REGULATORY RATES SUSTAIN CROP YIELDS AND C, N, P, AND K DYNAMICS IN A FRENCH CALCAREOUS SOIL

ABSTRACT

Recycling organic waste (OW) in agriculture can improve soil fertility and substitute chemical fertilizers depending on the OW and their treatment. The effects of OW have often been studied in simplified cropping systems to strengthen the observed effects. The objective of this study was thus to evaluate the long-term effects of different types of OW used at European regulatory rates on C storage, crop yields and N, P, and K dynamics. OW has been applied every 2 years at 170 kg N ha⁻¹ since 2001 in the long-term field experiment PROspective in northeastern France on a silty loam calcosol. The 5 types of OW included urban sewage sludge (SLU), green waste and SLU compost (GWS), municipal biowaste compost (BIO), farmyard manure (FYM), and composted FYM (FYMC). The control treatment (CON) did not receive any OW. All treatments were studied after applying (N+) or not applying (N-) mineral N fertilization at an annual optimal rate. Biowaste digestate was also applied after 2014 in N- treatments. OW application increased crop yield compared with the unfertilized control. Mineral N fertilizer partially substituted by OW allowed crop yield to be sustained compared with mineral fertilizer only, saving 18-54% mineral N fertilizer, 56-80% mineral P fertilizer and 14-76% mineral K fertilizer. No effects on crop grain N, P and K concentrations were found. The efficiency of OW to maintain SOC, total N, Olsen-P and exchangeable K contents in soils greatly varied with the type of OW. Except for SLU, the SOC stocks significantly increased from +2.9 to +7.0 t SOC ha⁻¹ for FYMC_N- and BIO_N-, respectively. SLU and digestate had the greatest N fertilizer replacement value (58% and 69%, respectively). N-leaching risk did not increase with OW application in the long term. For a positive ΔP of 100 kg ha⁻¹, Olsen-P increased by 2 mg P kg⁻¹ in the GWS_N- and SLU_N- treatments, whereas Olsen-P decreased in other treatments. A surplus of +100 kg ha⁻¹ ΔK raised the exchangeable K stock by 20 and 21 kg K ha⁻¹ in the FYM_N- and FYMC_N- treatments, respectively, whereas exchangeable K decreased in the BIO and GWS treatments. Our results highlight the ability of all tested types of OW to sustain crop yields in the long term when used at EU regulatory rates, while their effects on mineral fertilizer savings, SOC, soil mineral N, Olsen-P, and exchangeable K contents greatly varied according to the considered OW.

KEYWORDS

Organic waste recycling; Crop yields; Calcareous soil; Long-term field experiment; Soil dynamics

LIST OF ABBREVIATIONS

ANR: Apparent N recovery

BIO: Biowaste compost

DM: Dry matter

FYM: Farmyard manure

FYMC: Farmyard manure compost

GWS: Compost of green waste and urban sewage sludge

LTFEs: Long-term field experiments

NFRV: Nitrogen fertilizer replacement value

OW: Organic waste

PROC: Potentially residual organic carbon

SLU: Dehydrated urban sewage sludge

SMN: Soil mineral nitrogen

5.1 INTRODUCTION

Organic waste (OW) is derived from waste materials of industrial, agricultural and municipal origin. Due to the nutrient contents in OW, many studies have highlighted the advantages of OW application as a substitute for mineral fertilizers as an agroecological practice for sustainable agriculture (Wezel et al., 2014). The large variety of OW and treatment methods result in a large diversity of OW and their effects after OW application (Gómez-Muñoz et al., 2017).

A considerable number of studies have pointed out that long-term field experiments (LTFEs) are the most relevant and reliable way to evaluate soil-crop systems and predict future trends, since the cumulative effects of OW application on soil properties and crop yields can last for several years and might become measurable after several years (Diacono and Montemurro, 2010; Eghball et al., 2004; Wei et al., 2016). Based on LTFEs, many authors have reported that repeated application of OW enhances soil organic matter content (Peltre et al., 2012; Zavattaro et al., 2017) and soil N mineralization (Chalhoub et al., 2013), improves soil physical properties (Eden et al., 2017; Paradelo et al., 2019), and increases the abundance and activity of microbial biomass (Sadet-Bourgeteau et al., 2018). Moreover, OW spreading could supply nutrients and enhance their availability (Diacono and Montemurro, 2010; Weber et al., 2007). This improved soil fertility usually results in higher grain yields when OW and mineral fertilizers are combined in comparison to mineral fertilizers only (Wei et al., 2016, 2020; Zhang et al., 2020).

However, repeated applications of OW to cropland may also have negative effects, including increased greenhouse gas (GHG) emissions (Sánchez-Navarro et al., 2020) and heavy metal accumulation in soils (Guo et al., 2018; Michaud et al., 2020; Muhammad et al., 2020). Nitrate leaching may also be increased with OW, especially if it is applied in autumn on a crop with limited N uptake (e.g., winter wheat) (Nicholson et al., 2017; Thomsen, 2005).

Previous studies have made some attempts to assess the discrepancy in the effects among different types of OW after long-term application, including soil properties (Annabi et al., 2011; Lemming et al., 2019), C and N dynamics (Gómez-Muñoz et al., 2017; Noirot-Cosson et al., 2016) and microbial community composition (Poulsen et al., 2013; Sadet-Bourgeteau et al., 2018). However, some studies used excessive OW input and removed crop residues to strengthen the effects of OW (Gerzabek et al., 1997; Noirot-Cosson et al., 2016). Little is known regarding the long-term effects of different kinds of OW applied in usual agricultural practices, i.e., at a maximum rate of 170 kg N ha⁻¹ yr⁻¹ according to the European Union “nitrates” directive (Directive 91/676/EEC), crop residues not necessarily removed and adjustment of mineral fertilization. Finally, most studies have been based on LTFEs in noncalcareous soils with acidic to neutral pH (Chen et al., 2018), although the dynamics of soil nutrients can be strongly modified in calcareous soils (Amrani et al., 1999; Pilar Bernal et al., 1993), and calcareous soils

represent important areas worldwide (Holloway et al., 2001).

Thus, the objective was to study the effects of composted or raw OW repeatedly applied every 2 years for 18 years (9 applications) on crops and C, N, P and K dynamics in soils and to identify the characteristics of OW linked to the variability of OW effects. We focused more precisely on the effects on (1) crop yield and N, P, and K contents; (ii) soil chemical fertility (C, N, P, and K contents); and (iii) soil mineral N supply and risk of N losses. We used the PROspective field experiment located at the Colmar Experimental Centre of the French National Research Institute for Agriculture, Food and Environment (INRAE) located in northeastern France, which includes five different types of OW combined with two mineral N fertilization strategies: OW only (without supplementation with mineral N fertilization) and OW + optimal rate of mineral N fertilization.

5.2 MATERIAL AND METHODS

5.2.1 Site description

The LTFE PROspective is located at the Colmar Experimental Centre of the French National Research Institute for Agriculture, Food and Environment (INRAE), northeastern France (48°03'33" N, 7°19'42" E, altitude 200 m). The climate is semicontinental, with a mean annual precipitation of 559 mm received mostly between May and October and an average annual air temperature of 11.3°C. The daily climate data (air temperature and rainfall) were collected from a weather station located 500 m away from the experimental site. The soil is classified as Calcisol (with 125.5 g kg⁻¹ calcium carbonate in the plowed layer) according to the World Reference Base for Soil Resources (IUSS Working Group WRB, 2015). The first 120 cm presents a silty loam texture, without any coarse fragments, and corresponds to the depth explored by roots. The plowed (0–28 cm) layer contains 21% clay, 70% silt, and 9% sand on average. Before the beginning of the trial, the main topsoil physicochemical characteristics were as follows: pH (in water): 8.3; total N (TN): 1.4 g kg⁻¹; soil organic carbon (SOC): 14.3 g kg⁻¹; Olsen-P (i.e., NaHCO₃-extractable P): 31 mg kg⁻¹; and exchangeable K (cobaltihexamine): 269 mg kg⁻¹.

The experiment started in 2001 as a part of the SOERE-PRO network (https://www6.inra.fr/qualiagro_eng/Nos-partenaires/The-SOERE-PRO-network). The crop succession was a maize (*Zea mays*)-winter wheat (*Triticum aestivum*)-sugarbeet (*Beta vulgaris*)-spring barley (*Hordeum vulgare*) rotation since 2001 (except for 2003, when maize was sown instead of sugarbeet) with the same yearly crop in the different treatments. All aboveground crop residues were returned to the soil. Since 2014, mustard cover crops before spring crops (sugarbeet and maize) have been used. The experiment was conducted in conditions close to local agricultural practices in terms of doses of provided OW, agricultural equipment used, and without irrigation. Regular plowing to a 28 cm depth occurred each year in late autumn or winter. Pest, weed and

pathogen control relied on pesticides.

5.2.2 Design of the long-term field experiment

The 2-ha experimental field was divided into 60 plots (10 m × 9 m). All plots were separated by 10 m wide buffer strips to avoid cross contamination. There were 4 experiments arranged side by side (Figure. S1):

- Six OW_N- treatments in a complete randomized block design (4 replicates) =24 plots: 5 OW and a control without applying mineral N fertilizer (N-);
- Six OW_N+ treatments in a complete randomized block design (4 replicates) =24 plots: 5 OW and a control with optimal mineral N fertilizer (N+);
- Six SN treatments (no replicate) = 6 plots: 5 OW and a control under bare fallow (no crops);
- Six N treatments (no replicate) = 6 plots: cultivated without OW and with rotation of mineral N supplementation (2 plots without mineral N every year to determine apparent nitrogen recovery, see section 2.6).

The five OW treatments were as follows: (1) a dehydrated urban sewage sludge (SLU) derived from the SITEUCE wastewater treatment plant serving around 300,000 inhabitants, which was produced by extended aeration, precipitation of P with aluminum salt, and then centrifugal dehydration; (2) a compost of green waste and SLU (GWS) derived from the abovementioned wastewater treatment plant co-composted in a forced aeration composting system; (3) a biowaste compost (BIO) made from the home-sorted organic fraction of municipal solid waste co-composted with green waste; (4) a farmyard manure (FYM) made from a mix of dairy cow feces and urine with cereal straw; and (5) a farmyard manure compost (FYMC), resulting from 2 months of composting in open air on a concrete platform of the abovementioned farmyard manure. The sixth treatment corresponded to a control without any OW application (CON). Each OW came from one to four different plants with similar processes according to the year. The two levels of N supplementation were (1) no mineral N supplementation (N-) from 2000 to 2015 and digestate application after 2015 and (2) optimal mineral N supplementation (N+), which attempted to obtain economically optimal yields. In the N+ treatments, ammonium nitrate or urea was applied once or twice every year for each treatment. The quantity of mineral N applied was adjusted in each treatment as a function of the quantity of mineral N in the soil in early spring. In 2007, 2011 and 2015, potassium sulfate and triple superphosphate were applied in the N+ treatments.

In 2001, 2003, 2005, 2007, 2009, 2011 and 2013, the same amount of OW (approximately 170 kg N ha⁻¹, maximal annual amount allowed by EU legislation) was applied in early spring before maize or sugarbeet. In 2014 and 2016, the application

period changed to summer before the sowing of the catch crop and after the harvest of cereals. The fertilization strategy in the N- treatments was changed after 2014 to reach equivalent yields in comparison to N+ treatments with only OW. The amounts of OW in the N- treatment were thus adjusted at each application and for each treatment based on the N requirements of the spring crop and of the characteristics of each OW (N content and potentially available N). Moreover, after 2015, a raw digestate was also applied every year if needed to supply the additional required amount of available N and to reach the same economically optimal yield as in the N+ treatment. The digestate came from an anaerobic digestion plant and was composed of approximately 60% digested food wastes, 20% digested slurry and manure and 20% digested agrofood industrial waste. The changes in the experiment design was motivated by the already large amount of information brought by the N- treatments in 2013 and the drop in soil fertility and crop yields in this part of the experiment.

Details of crop species, duration of cultivation period, soil sampling dates, and OW and N fertilizer application dates are shown in Table S1. The detailed amounts of applied OW, digestate and mineral fertilizer are presented in Tables S2, S3, S4 and S5.

5.2.3 Harvests and plant analysis

Plants were sampled every year immediately before mechanical harvest to determine total aboveground biomass and grain and sugarbeet root yields. Grain and crop residues were analyzed for nutrient contents. After crop harvest, grain and crop residues were oven dried at 103°C to a constant weight for the analysis of dry matter and nutrient contents. The N, P and K uptake was calculated by multiplying the yields and N, P, and K contents of all harvested crop components. Since crop residues were laid on the soil every year, the difference ΔN , ΔP and ΔK , between cumulative sum of applied and harvested N, P and K, respectively, were calculated every two years:

$$\Delta N = N_{\text{applied}} - N_{\text{harvested}} \quad (\text{Equation 5-1})$$

$$\Delta P = P_{\text{applied}} - P_{\text{harvested}} \quad (\text{Equation 5-2})$$

$$\Delta K = K_{\text{applied}} - K_{\text{harvested}} \quad (\text{Equation 5-3})$$

where N_{applied} , P_{applied} and K_{applied} are the cumulative sum annual of N, P and K applied (as OW, mineral fertilizer and digestate), respectively, and $N_{\text{harvested}}$, $P_{\text{harvested}}$ and $K_{\text{harvested}}$ are the cumulative sum of annual N, P and K uptake in harvested parts of the crops, respectively.

5.2.4 Soil sampling and analysis

Soil from the plowed layer (0–28 cm) was sampled in each experimental plot and analyzed before the beginning of the experiment (2000) and every two years (2002, 2004, 2006, 2008, 2010, 2012, 2014, 2016 and 2018) after crop harvest and before OW application. Soil analyses were carried out by the Laboratoire d'analyses des sols of

INRAE (LAS, Arras, France) using French standardized analytical methods (<https://www.afnor.org/>).

Particle size analysis of soil was performed after organic matter mineralization by H₂O₂ and dispersion with Na-hexametaphosphate (NF X31-107). Total C and N were determined by dry combustion (NF ISO 10694 & NF ISO 13878). The cation exchange capacity (CEC) was determined using the cobaltihexamine method (NF X 31-130). Soil pH was measured in a 1:5 soil:liquid suspension (v/v) (NF ISO 10390). Available P was estimated by sodium bicarbonate extraction (NF ISO 11263), as proposed by Olsen (Olsen, 1954). Exchangeable K were analyzed by ICP-AES. Soil bulk density was determined with the soil core method (Blake and Hartge, 1986) every 2 or 3 years with cores of known volume (250 cm³ or 500 cm³).

Due to the influence of carbonates on the C content analysis, the organic C stock data were unreliable in our study. Because the C/N ratio of each treatment was stable, we used the organic N stocks and a constant C/N ratio to compute the organic C stocks for each year. C stocks computed with the measured C contents are shown in Figure. S2.

From soil nutrient contents and soil bulk density (BD), nutrient stocks in the 0-28 cm layer were computed. As bulk density did not change significantly across treatments and years (standard deviation equal to 0.1), we used a constant soil bulk density of 1.3.

Mineral N stocks in soil were measured after KCl extraction, then N-NO₃ was measured by NF ISO 11732 and N-NH₄ was measured by NF ISO 13395. Soil mineral N was analyzed twice to thrice yearly, at the beginning of winter before the drainage period, at the end of winter after the drainage period and before maize sowing or after wheat and barley harvests (every two years), in four soil layers (0-30, 30-60, 60-90, and 90-120 cm). However, due to a mistake in the experimental protocol, data from 2003 to 2008 were unusable.

5.2.5 Computation of carbon input

The cumulative C input to soil was calculated, corresponding to OW application (C_{OW}), digestate input ($C_{digestate}$), returned crop residues (C_S), C in root residues (C_R) and extraroot (root exudates) carbon (C_E).

$$C_{input} = C_{OW} + C_{digestate} + C_S + C_R + C_E \quad (\text{Equation 5-4})$$

Due to the lack of measurement data on crop root C and extraroot C, relative C allocation coefficients were used to calculate belowground C inputs from crop residues (Bolinder et al., 2007; Clivot et al., 2019).

$$C_R = R_R/R_S \times C_S \quad (\text{Equation 5-5})$$

$$C_E = R_E/R_S \times C_S \quad (\text{Equation 5-6})$$

where R_R is the relative root C allocation coefficient, R_E is the relative extraroot C allocation coefficient, and R_S is the relative aboveground residue carbon allocation coefficient. The detailed values of the relative C allocation coefficients in different crops are presented in Table S6.

Humification coefficients (h), the fraction of organic matter converted to humified organic matter in the year following application, were introduced to assess the potential contribution of crop residues and OW input to soil organic carbon. This potentially residual organic carbon ($PROC$), which contributed to soil organic C, was estimated with the following formula:

$$PROC = h_{OW} \times A_{OW} + h_{digestate} \times A_{digestate} + h_{crop} \times A_{crop} \quad (\text{Equation 5-7})$$

where A_{OWP} , $A_{digestate}$ and A_{crop} are the C inputs of OW, digestate and crop aboveground and belowground residues returned to the soil, respectively, and h_{OW} , $h_{digestate}$ and h_{crop} are the humification coefficients of OW, digestates and crop residues, respectively. The I_{ROC} (Table 5-1) was used as the h value for OW and digestate, as suggested by Levavasseur et al. (2020). The indicator of residual organic C (IROC) was defined as a predictor of C remaining from OW after long-term incubation of OW with soil under laboratory-controlled conditions (Lashermes et al., 2009). It was determined from biochemical fractions of the OW and the proportion of C mineralized during a very short incubation (3 days). h values of different crop residues were based on Clivot et al. (2019). The detailed values of the humification coefficients are presented in Table S6.

Table 5-1 Mean characteristics \pm standard deviations of organic waste products applied 9 times in the experiment from 2001 to 2018. For digestate, there were 4 applications from 2015 to 2018. For each year, 3 samples were analyzed for each OW. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$.

OW	SLU	GWS	BIO	FYM	FYMC	Digestate
DM (% of fresh matter)	18.2 \pm 1.7 b	55.1 \pm 7.7 a	61.0 \pm 12.0 a	20.9 \pm 3.3 b	19.6 \pm 2.5 b	6.8 \pm 0.9 c
pH (water)	7.2 \pm 0.6 c	7.5 \pm 0.4 c	8.4 \pm 0.4 b	9.5 \pm 0.3 a	9.4 \pm 0.3 a	8.6 \pm 0.3 b
Organic C (g kg ⁻¹ DM)	368 \pm 28 ab	293 \pm 46 c	252 \pm 23 d	398 \pm 18 a	351 \pm 27 b	378 \pm 25 ab
Total N (g kg ⁻¹ DM)	59.5 \pm 4.6 b	26.3 \pm 4.6 c	20.4 \pm 3.2 c	24.7 \pm 4.6 c	25.6 \pm 1.7 c	113.9 \pm 16.0 a
NH ₄ ⁺ (g kg ⁻¹ DM)	16.3 \pm 7.6 b	4.0 \pm 1.5 b	0.8 \pm 0.7 b	1.7 \pm 2.0 b	0.4 \pm 0.2 b	59.4 \pm 12.4 a
NO ₃ ⁻ (g kg ⁻¹ DM)	0.01 \pm 0.01 a	0.45 \pm 0.63 a	0.11 \pm 0.23 a	0.46 \pm 0.61 a	0.12 \pm 0.11 a	0.04 \pm 0.01 a
C: N	6.3 \pm 0.3 d	11.3 \pm 2.6 c	12.6 \pm 1.5 bc	16.9 \pm 4.0 a	14.1 \pm 1.4 b	3.4 \pm 0.4 e
Total K (g kg ⁻¹ DM)	5.2 \pm 1.0 e	11.1 \pm 3.9 d	18.1 \pm 2.9 c	31.3 \pm 4.6 b	35.6 \pm 6.6 b	49.7 \pm 9.3 a
Total P (g kg ⁻¹ DM)	29.3 \pm 1.7 a	15.9 \pm 5.1 b	4.8 \pm 0.9 d	6.3 \pm 1.5 cd	8.2 \pm 1.7 c	14.3 \pm 1.5 b
I_{ROC} (%)	49.4 \pm 9.6 b	72.3 \pm 4.5 a	70.1 \pm 8.7 a	57.0 \pm 6.8 b	67.8 \pm 4.0 b	56.3 \pm 4.6 b

5.2.6 Computation of the N fertilizer replacement value

The N fertilizer replacement value (*NFRV*) was used to estimate the N contribution of OW. Generally, the *NFRV* can be calculated with both DM yield and N yield, but using N yield is considered to provide a more reliable prediction (Middelkoop and Holshof, 2017). To calculate the *NFRV* of SLU, GWS, BIO, FYM and FYMC, we used the N-experiment from 2001 to 2014 (before digestate application). For the *NFRV* of digestate, data from 2015 to 2018 were used. The *NFRV* in our study was calculated by N uptake based on apparent N recovery (ANR):

$$ANR = (Nuptake_{treatment} - Nuptake_{unfertilized}) / Total\ N\ applied \quad (Equation\ 5-8)$$

$$NFRV_{OWs}(\%) = ANR_{OWs} / ANR_{CON_{N+}} \times 100 \quad (Equation\ 5-9)$$

where $Nuptake_{treatment}$ is the cumulative N uptake of each treatment; $Nuptake_{unfertilized}$ is the cumulative N uptake of the yearly unfertilized control treatment (CON_N- before 2014 and unfertilized plots among N1-N6 after 2015); and $ANR_{CON_{N+}}$ is the ANR of the mineral N addition treatment (CON_N+).

5.2.7 Statistical analysis

Statistical analyses were performed with R software (Version 3.6.2). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$ level of significance) was used for separation of means. In addition, simple linear regression was used to detect the relationship between soil stocks, input and balance for C, N, P and K.

5.3 RESULTS AND DISCUSSION

5.3.1 Characteristics of OW

The mean characteristics of OW are shown in Table 5-1. All OW had pH values greater than 7, and FYM and FYMC showed the highest pH, whereas SLU and GWS presented the lowest pH. In comparison to other OW, SLU and digestate had lower dry matter contents and larger total N and mineral N contents (mainly NH_4^+), therefore resulting in a lower C/N ratio (3.4-6.3). SLU was characterized by the greatest total P content, followed by digestate and GWS. K decreased in the following order: Digestate = FYMC > FYM > BIO > GWS > SLU. The index of residual organic C (I_{ROC}) was used to describe the stability of C after application to soil (Lashermes et al., 2009). SLU, FYM, FYMC and digestate were generally richer in C content (in %DM) compared with GWS and BIO, whereas they had a lower I_{ROC} value (49.4-67.8%), indicating that C in SLU, FYM, FYMC and DIG was less stable than in GWS and BIO.

5.3.2 Crop yields

The mean harvested biomass of maize, wheat, barley, and sugarbeet was 8.9, 4.0, 4.3,

and 17.3 t ha⁻¹ yr⁻¹ for the N- experiment and 9.4, 6.4, 4.6, and 16.3 t ha⁻¹ yr⁻¹ for the N+ experiment, respectively (Figure 5-1a). A similar increase in residue biomass with mineral N fertilization was also observed, with residue biomass of 8.6, 4.3, 4.6 and 6.8 t ha⁻¹ yr⁻¹ for the N- experiment for maize, wheat, barley, and sugarbeet and 8.9, 7.1, 5.3 and 7.5 t ha⁻¹ yr⁻¹, respectively, in the N+ experiment (Figure 5-1b). Mineral fertilization thus significantly increased grain biomass, as well as residue biomass except for sugarbeet, mainly caused by a sufficient nutrient supply with OW. However, there was no significant influence of mineral N or OW applied on the harvest index (HI) in all experiments (Table S7), which indicates that OW applied did not impact the physiological efficiency of plants in converting to grain yield (Fosu-Mensah and Mensah, 2016).

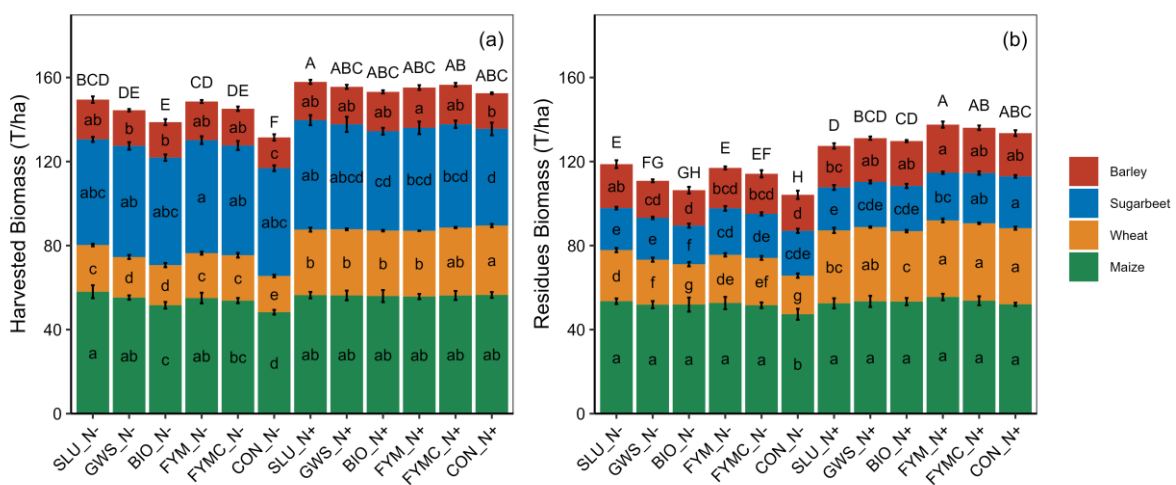


Figure 5-1 Cumulative harvested biomass (a) and aboveground residue biomass (b) (DM) of barley, sugarbeet, wheat and maize over 18 years (2001-2018) under the 12 treatments (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: dairy farmyard manure; FYMC: FYM after composting; CON: control; and N+: optimal dose of mineral N fertilization and N-: no mineral N fertilization but digestate application from 2015). Error bars are the standard deviations (four replicates).

In the N- experiments, OW application significantly increased the grain biomass of maize, wheat and barley compared with the control without mineral fertilizer (CON_N-). However, the grain biomass was significantly lower than that in the control with mineral N fertilization (CON_N+), especially for wheat (one year after OW application) and for maize in the BIO_N- treatment. The positive effects of OW on crop yields might be mainly based on the N, P, K and secondary nutrient (calcium, magnesium, sulfur, etc.) supplies from OW. Moreover, it has been reported that the soil structure and availability of soil water improvement (Annabi et al., 2011; Paradelo et al., 2019) and soil microbial activity enhancement (Bonanomi et al., 2020; Liu et al., 2010) could also contribute to yield increases. After 2014, digestates were applied in OW_N- treatments. Except for wheat, the combined OW and digestates resulted in even higher yields than OW_N+. Moreover, CON_N-, where digestates have been applied every year since 2014, reached the same yield as CON_N+ in maize and barley and was even higher in wheat and sugarbeet (Table S8). These results indicate the large potential of mineral N substitution

by digestates, as suggested by several authors (Riva et al., 2016; Sigurnjak et al., 2017). It is worth noting that to reach the optimal yields for each treatment, we applied excessive OW and digestates after 2014 in OW_{N-} treatments that no longer respect EU legislation.

In the N₊ experiments, compared with the control with mineral N fertilization (CON_{N+}), the OW_{N+} treatments showed almost no difference in maize, barley and sugarbeet (except a higher crop yield for sugarbeet in SLU_{N+} and a higher crop yield for barley in FYM_{N+}), whereas the OW treatments, except FYMC_{N+}, decreased the grain biomass of wheat. All treatments reached the same total grain biomass in 18 years, which indicates that long-term mineral fertilizer partial substitution with OW (OW_{N+}) sustained crop yield to the levels of mineral fertilizer only (CON_{N+}). Our results are consistent with other studies (Li et al., 2020; Lv et al., 2020; Xin et al., 2017) that reported that the partial substitution of mineral N by OW could reach the same crop yields. It is worth noting that the different substitution strategies caused inconsistent results of OW on yield. In our study, the quantity of mineral N was adjusted for each N₊ treatment as a function of the quantity of soil mineral N in early spring, whereas in many studies, the same mineral N was applied among treatments. For example, Wei et al. (2020) carried out a meta-analysis to evaluate the effect of substituting mineral N fertilizer with the same amount of N application and found a 4.22% maize yield increase among 133 studies. Moreover, different soil characteristics, inherent soil fertility, climatic conditions, experimental durations, fertilization regimes and fertilizer application rates could determine the effect of OW application (Chen et al., 2018; Du et al., 2020; Liu et al., 2021; Wortman et al., 2017; Zhang et al., 2020).

In our study, OW was applied every two years; therefore, the available N from OW mostly benefited plants in the first-following year (maize or sugarbeet). These results are supported by the fact that the yield of wheat sown in the second year after OW application was significantly reduced in the OW_{N-} treatments in comparison to the CON_{N+} treatment, whereas the maize and sugarbeet grain biomass was nearly similar for every OW treatment (except BIO_{N-} treatment).

5.3.3 N, P, K concentrations in crop harvests

The mean N concentrations were 12.5 g kg⁻¹ DM, 17.7 g kg⁻¹ DM, and 15.7 g kg⁻¹ DM for maize, wheat, and barley grains, respectively, and 5.5 g kg⁻¹ DM for sugarbeet roots (Figure. S3). Grain N content is a critical index for crop production, especially for wheat, which needs sufficient protein for bread making. Mineral N application significantly increased grain N concentrations in all kinds of crops, whereas OW types had no effect on grain N concentrations (Table S9). A possible explanation for the decreased N content in the OW_{N-} treatments was the lack of synchrony between the N supply by OW and the N demand of crops. Similar results were also reported by Saha et al. (2008), who found that N applied in mineral form caused the highest grain protein content.

Before digestate application in 2015, the N concentrations in barley and wheat of all

treatments in the N+ experiment were significantly higher than those in the N- experiment ($P < 0.05$). However, after digestate application in 2015, the N- treatments reached the same N concentration for barley and wheat as the N+ treatments. Moreover, in the last 4 years, CON_N-, in which most of the digestate was applied, reached the highest N concentration in wheat, barley and sugarbeet. These results highlight the potential of digestate for a good crop N supply, in line with the study of Barłóg et al. (2020), who found that digestate resulted in a higher N content in barley.

The mean P concentrations were $2.7 \text{ g kg}^{-1} \text{ DM}$, $3.9 \text{ g kg}^{-1} \text{ DM}$, and $3.7 \text{ g kg}^{-1} \text{ DM}$ for maize, wheat, and barley grain, respectively, and $1.0 \text{ g kg}^{-1} \text{ DM}$ for sugarbeet roots (Figure. S4). These values were consistent with those reported in normalized references in France (COMIFER, 2007) and corroborated those found in other experiments (Cadot et al., 2018; Tang et al., 2008). For example, the grain P content is 2.8 g kg^{-1} for wheat and barley and 2.6 g kg^{-1} for maize in the COMIFER table. Regardless of the crops, no significant differences were observed in grain P concentration depending on the types of OW applied. However, N fertilizer application decreased the P concentration of wheat and barley. OW_N- increased the P concentration compared with OW_N+, perhaps due to P dilution in the increasing grain biomass (Cadot et al., 2018).

The mean K concentrations were $3.6 \text{ g kg}^{-1} \text{ DM}$, $4.6 \text{ g kg}^{-1} \text{ DM}$, and $5.7 \text{ g kg}^{-1} \text{ DM}$ for maize, wheat, and barley grain, respectively, and $7.0 \text{ g kg}^{-1} \text{ DM}$ for sugarbeet roots (Figure. S5). No evidence showed that OW or chemical fertilizer impacted the crop grain K concentration (Table S9). These results were in line with the study of Jiang et al. (2018), who analyzed 60 experimental sites for maize in China and reported that the rates of K fertilizer applied did not significantly increase maize grain K concentrations.

5.3.4 Dynamics of soil organic C stocks

5.3.4.1 Carbon input

The total C input and potentially residual organic C input (*PROC*) from crop residues, OW and digestate in the different treatments are shown in Figure 5-2. More than 70% of the total C input was from crop residues, and SLU_N+, which received the lowest C from OW, relied mostly on crop residue C input (91.0%), whereas only 71.3% of the total C input was from crop residues in FYM_N-. *PROC* also mainly relied on crop residue C input but to a lesser extent: 54.4% of *PROC* was from crop residues in BIO_N-, whereas 85.6% of *PROC* was from crop residue C in SLU_N+. We emphasized that the variations in C input in the different treatments were attributed to different C inputs from OW but also to differences in crop residues produced and incorporated into the soil. These results confirm that long-term applications of OW may increase soil organic C by the addition of organic matter to soil both directly and indirectly by increasing crop residues (Kundu et al., 2007).

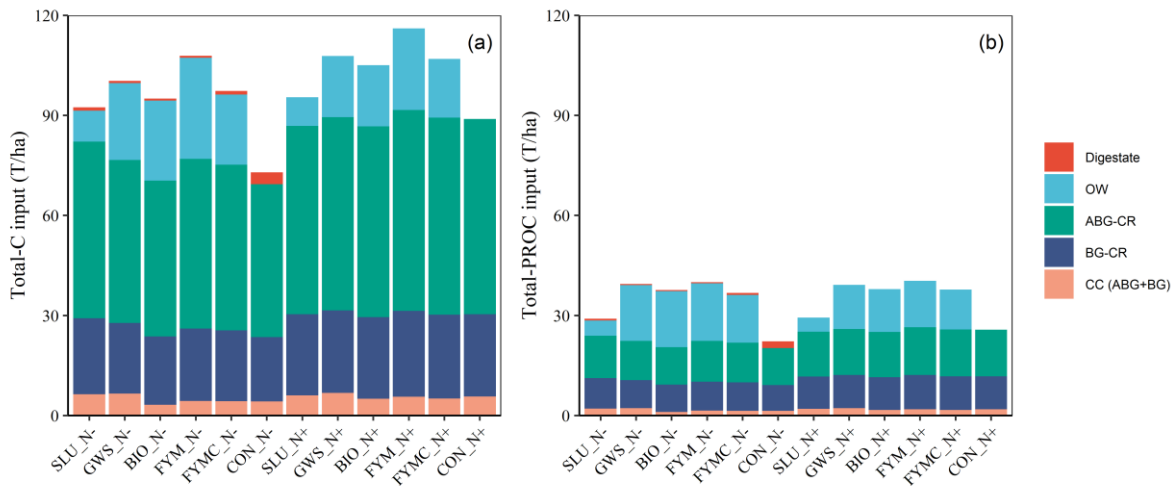


Figure 5-2 The total C input and total PROC input from different sources (SLU: dehydrated sludge; GWS: green waste and sewage sludge compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; CON: control; N+: optimal mineral N fertilization; N-: no mineral N fertilization; ABG-CR: aboveground crop residues; BG-CR: belowground crop residues; and CC: cover crops).

5.3.4.2 Soil organic C dynamics

The initial SOC stocks varied between 50.1 and 55.1 t ha⁻¹ in 2000, with a statistically significant difference among treatments. Repeated OW applications significantly influenced SOC stocks, and the interannual change in the SOC stocks exhibited considerable fluctuations over 18 years (Figure 5-3). The SOC stocks in bare soil showed a dramatic decrease even if the same quantities of OW were applied. SOC stocks changes ranged from -9.3 to -15.4 t ha⁻¹ during 18 years, equivalent to 17% to 31% of initial C stock. These results indicating the important role of crop residues on C stocks and highlighted that OW applied at this rate could not maintain the SOC stocks without the return of crop residues.

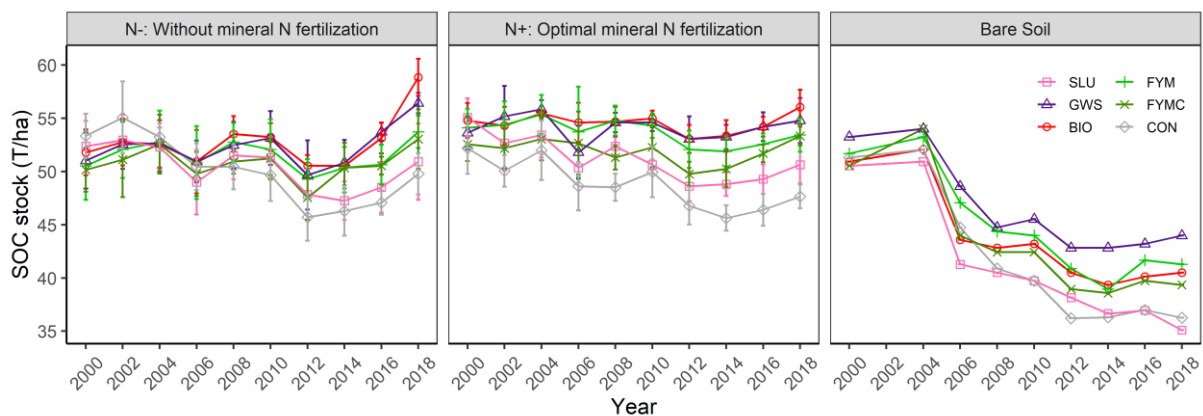


Figure 5-3 Dynamics of SOC stocks in the plowed layer under the different OW applications and CON without mineral N fertilization (left), with optimal mineral N fertilization (center) and in bare soils (right) (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control). Error bars are the standard deviations (four replicates).

During 18 years, the SOC stocks in GWS_N-, BIO_N-, FYM_N- and FYMC_N- increased by +5.4, +7.0, +3.2 and +2.9 t ha⁻¹, respectively, whereas they decreased in SLU_N- and CON_N- by -1.4 and -3.6 t ha⁻¹, respectively. In the N- experiment, all OW application treatments, including SLU_N-, showed a significantly higher SOC stock change relative to the control treatments ($P < 0.05$) (Table S10). In the N+ experiment, the SOC stocks increased in the GWS_N+, BIO_N+ and FYMC_N+ (1.2, 1.3 and 0.8 t C ha⁻¹, respectively), while they decreased in the SLU_N+, FYM_N+ and CON_N+ treatments (-4.4, -0.7 and -4.5 t ha⁻¹, respectively). Only SLU_N+ stood out and showed a change in SOC stocks not significantly different than the control treatment (Table S10). These results indicated a positive effect of realistic OW applications on SOC stocks, which was in accordance with previous studies (Bhattacharyya et al., 2010; Montemurro et al., 2006).

The changes in SOC stock between 2000 and 2018 showed a positive relationship with total C input (Figure 5-4). These results are in line with the literature that highlighted that increased total C input is the main driver of increased SOC stocks (Chenu et al., 2019). However, the correlation of SOC change was higher with *PROC* than with total C input, which suggested that *I_{ROC}* is a valuable indicator in estimating the contribution of OW C to SOC (Figure 5-4). This confirmed that the C proportion contributing to SOC storage in the long term differed among OW and between OW and crop residues, as suggested by various authors (Gerzabek et al., 1997; Levavasasseur et al., 2020). Different humification coefficients of OW leading to different *PROC* inputs might be one of the explanations for different SOC stocks among different types of OW; the BIO and GWS composts showed the highest humification coefficients (70% and 72%, respectively), whereas SLU showed a lower humification coefficient (49%). Several studies have also confirmed that SLU was more easily degradable and therefore had less effect on SOC storage than other types of OW (Rudrappa et al., 2006; Triberti et al., 2008; Zavattaro et al., 2017). The humification coefficients for all OW were higher than those of crop residues (Table 1 and Table S6).

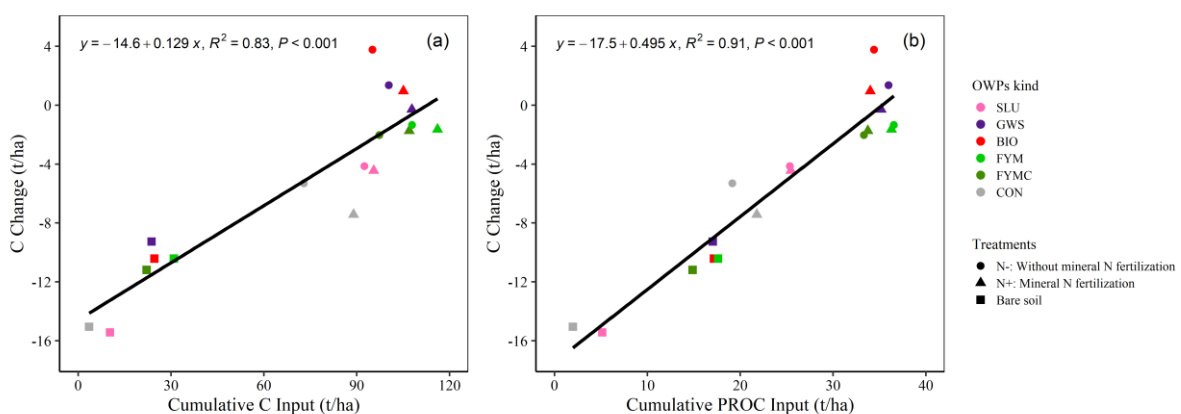


Figure 5-4 The relationship between cumulative C input, cumulative PROC input and SOC change in PROspective after 18 years (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: FYM after composting; and CON: control).

5.3.5 Soil N and mineral N

Soil mineral N stocks (SMN) in the 0-120 cm layer in early spring indicate the N supply for the next crop season. N fertilizer requirements in the N+ treatments were computed as the N demand for the target yield of specific crops minus the measured SMN in early spring, in line with local legislation. The differences in SMN in early spring were not significant during the first year after OW application (before maize or sugarbeet plants) among all treatments, whereas the SMN of SLU_N- and SLU_N+ was significantly higher than that of the CON_N+ and CON_N- treatments for the second year (Table 5-2).

Table 5-2 Mean soil mineral N stocks (0-120 cm) in early spring and late autumn for the 1st (year of OW application) and 2nd years (year after OW application). Values with the same lowercase letters in a column are not significantly different at $P < 0.05$.

Treatment	SMN stocks early spring (kg N year ⁻¹)		SMN stocks late autumn (kg N year ⁻¹)	
	1st year	2nd year	1st year	2nd year
	SLU_N-	68±16 a	125±52 ab	44±12 a
GWS_N-	53±13 a	101±24 abc	34±9 a	44±9 a
BIO_N-	53±13 a	89±22 abc	33±8 a	44±11 a
FYM_N-	63±18 a	82±14 bc	34±7 a	52±14 a
FYMC_N-	59±12 a	92±23 abc	36±9 a	51±17 a
CON_N-	59±25 a	66±20 c	30±9 a	63±39 a
SLU_N+	77±31 a	128±39.8 a	45±16 a	59±19 a
GWS_N+	72±18 a	101±24 abc	46±14 a	70±43 a
BIO_N+	80±27 a	96±26 abc	44±20 a	71±36 a
FYM_N+	80±22 a	86±19 abc	44±13 a	74±41 a
FYMC_N+	83±25 a	91±15 abc	45±11 a	79±46 a
CON_N+	79±28 a	79±23 c	47±14 a	80±35 a

Since the total cumulative yield was the same for every treatment in the N+ experiment, we could calculate the synthetic fertilizer savings for each OW by computing the difference in synthetic fertilizer input for OW_N+ with CON_N+ (Table 5-3). SLU_N+ showed the largest savings in mineral fertilizer N, saving 57.3 kg N ha⁻¹ per year, followed by FYM_N+, BIO_N+, FYMC_N+ and GWS_N+. Both the SLU_N+ and GWS_N+

treatments saved the most P₂O₅ fertilizer, with 23 kg P₂O₅ ha⁻¹ savings per year, whereas they saved only 6 and 16 kg K₂O ha⁻¹ per year, respectively. FYM_N+ and FYMC_N+ resulted in the largest K₂O savings, with 30 kg K₂O ha⁻¹ per year, in line with the high K contents of FYM and FYMC.

Table 5-3 Mean mineral fertilizer saved based on the N+ experiment and N fertilizer replacement value (NFRV) based on the N- experiment for the different types of OW.

OW	Mineral fertilizer saved (kg yr ⁻¹ ha ⁻¹)			NFRV (%)
	N	P ₂ O ₅	K ₂ O	
SLU	57.3	23.1	5.7	58
GWS	17.6	23.1	16.2	30
BIO	22.4	16.1	26.7	21
FYM	27.4	18.4	30.5	35
FYMC	18.8	17.7	30.5	32
Digestate	-	-	-	69

Based on the N- treatments, the N fertilizer replacement values (NFRVs) of SLU, GWS, BIO, FYM, FYMC and digestate were 58, 30, 21, 35, 32 and 69%, respectively (Table 5-3). It is well known that NFRVs are highly related to the content and form of N in materials, and higher mineral N and easily mineralizable N in materials would likely provide more plant-available N or a higher NFRV (Ashekuzzaman et al., 2021; Berge et al., 2016; Hijbeek et al., 2018). Digestate and SLU, which contained more mineral N, resulted in higher NFRVs, while BIO had the lowest NFRV among all types of OW. The lowest NFRV in BIO could be attributed to both low mineral N content and a low mineralization potential of organic N (Levavasseur et al., 2021).

We assumed that SMN stocks in late autumn indicated N-leaching potential, as has been suggested by several authors (Mitchell et al., 2001; Myrbeck et al., 2012; Zhao et al., 2020). No significant difference was detected among all treatments in mineral N content, either in the first or second year after application. Although a lack of synchrony between the N supply from OW and the N demand of crops might cause excess N leaching in organic systems (Kirchmann and Bergström, 2001; Scheller and Vogtmann, 1995; Sørensen, 2004), no statistically significant differences were found between treatments in our study. These results highlighted that the application of OW did not increase the risk of N leaching in our study, as shown in other studies (Doltra et al., 2011; Knudsen et al., 2006; Pandey et al., 2018). On the one hand, it was reported that the application of organic fertilizer stabilized the soil structure, slowed N migration, and effectively reduced fertilizer N leaching (Diacono and Montemurro, 2010). Furthermore, after the change in the experimental design in 2014 (OW applied in

summer instead of the end of winter and cover crop planted after application), SMN stocks in late autumn were still not significantly different in the OW treatments in comparison to the control treatments (Table S11). The inclusion of cover crops since 2014 has thus mitigated N leaching, which could have otherwise increased with the change in the OW application period; this effect of cover crops is consistent with previous studies (Quemada et al., 2013). Notwithstanding that no statistically significant differences were found between treatments, the average mineral N content of OW_N+ treatments in late autumn (57.5 kg ha^{-1}) were significantly higher than OW only treatments (42.4 kg ha^{-1}) ($P < 0.05$), which suggests a higher leaching potential with combined application of OW and mineral N compared with OW only.

The cumulative ΔN was calculated as the difference between N applied as OW, mineral N fertilizer and digestate and N exported at crop harvests (Figure. S6). A significant positive linear correlation was found between the ΔN and soil total N change in the N- and N+ experiments (Figure 5-5). It is worth noting that the slope of regression in the OW treatments was much higher than that in the OW_N+ treatments. This indicates that more N application was needed to keep soil N stable in the N+ experiment and that mineral N was less efficient than organic N to maintain or increase SON stocks. In addition, as described by Bhattacharyya et al. (2010), the strong positive linear relationships between the N balance and soil N change suggest that higher SON stocks could be reached with higher N balance.

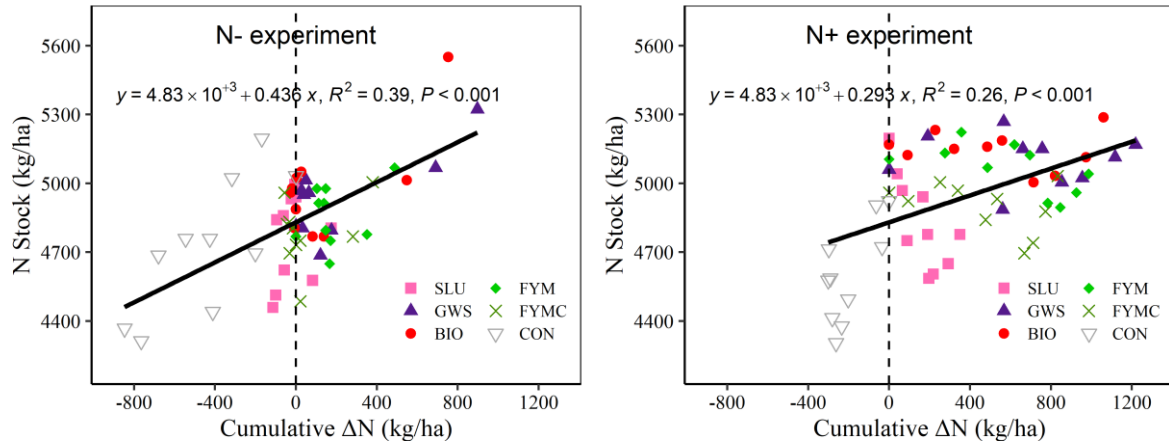


Figure 5-5 Relationship between cumulative ΔN and N stock in the soil plowed layer for the different CON and OW treatments in the N- and N+ experiments during the 18 years of experimentation (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control).

5.3.6 Olsen-P dynamics vs cumulative ΔP

In 2000, before any OW application, the average Olsen-P was significantly different in the N+ experiment; FUM_N+ had the highest Olsen-P ($129 (\pm 5) \text{ kg ha}^{-1}$); whereas FYMC_N+ had the lowest Olsen-P ($99 (\pm 6) \text{ kg ha}^{-1}$); and whereas no significant differences were measured across the N- experiment. The Olsen-P concentration

decreased over time for BIO_N+, FYM_N+, FYMC_N+, CON_N+, BIO_N- and CON_N-, whereas it slightly increased for SLU_N- and GWS_N- (Figure. S7). The cumulative ΔP was calculated as the difference between P applied as OW, mineral P fertilizer and digestate and P exported at crop harvests. In both the N- and N+ experiments, the GWS and SLU treatments, which received the most P input, resulted in the highest ΔP , whereas CON with the lowest P input resulted in the lowest ΔP . This ΔP increased after 2015 after the increase in the OW rate and digestate application in the N- experiment.

To account for differences in cumulative ΔP across treatments, we determined the relationship between Olsen-P stocks in the plowed layer vs cumulative ΔP for all treatments (Figure 5-6). For positive cumulative ΔP values, the slope of the linear regressions represents the amount of additional P required above that removed by the crop to raise the soil P status. Negative cumulative ΔP values represent the amount of P exported in harvests to decrease soil Olsen-P.

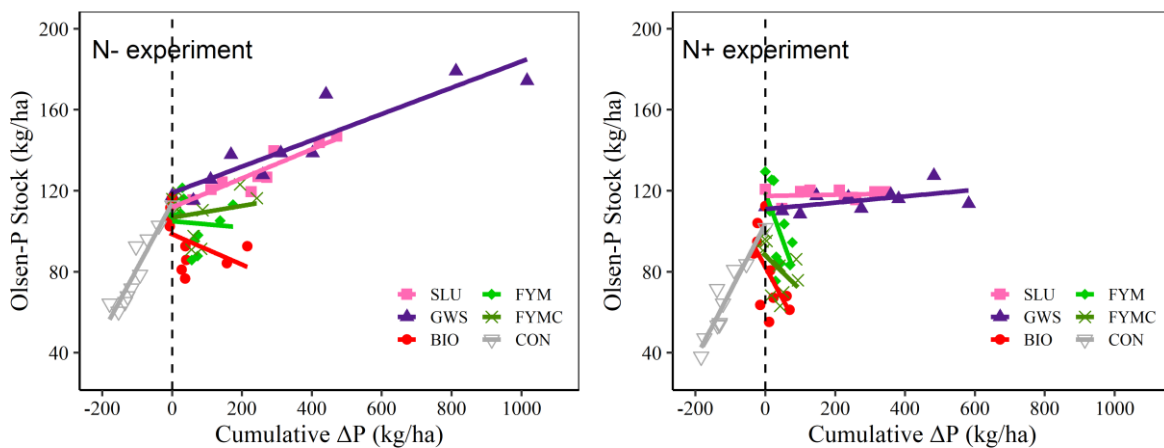


Figure 5-6 Relationship between cumulative ΔP and Olsen-P stock in the soil plowed layer for the different CON and OW treatments in the N- and N+ experiments during the 18 years of experimentation (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control).

The regression slopes show that a deficit of $-100 \text{ kg P ha}^{-1}$ in the cumulative P balance decreased Olsen-P by -33 and -35 kg ha^{-1} (equivalent to -9 and -10 mg P kg^{-1} soil, respectively) in the control treatment of the N- and N+ experiments, respectively (Table 5-4). Therefore, assuming that all P exported by harvesting crops came from the plowed layer, the Olsen-P method only extracted approximately 1/3 of the plant-available P in this calcareous soil. It is rather a high value in comparison with results observed in many soils in LTFEs. For example, Messiga et al. (2010) reported that Olsen extraction only extracted approximately 12% of the plant-available P in a slightly alkaline, sandy loamy soil (Iuvic Arenosol). At five sites across different soil types (two Calcaric Cambisols, a Haplic Luvisol, a calcaric Regosol, and an Eutric Cambisol) in China, Tang et al. (2008) found a decrease of $-3.4 \text{ mg Olsen-P kg}^{-1}$ on average for a deficit of $-100 \text{ kg P ha}^{-1}$, equivalent to 9% considering a plow layer depth of 0.20 m and bulk density of 1.3. In some cases, the soil Olsen-P concentration did not change significantly for increasingly

negative values of the ΔP , as reported by Selles et al. (1995) in a 24-yr crop rotation experiment conducted on a silt loam (Aridic Haploboroll) in southern Saskatchewan (Canada).

Table 5-4 Cumulative ΔP and associated variation in Olsen-P stock in the soil plowed layer after 18 years of experimentation.

Treatments	Olsen-P stock vs cumulative ΔP regression			
	Intercept	Slope	P-value	R ²
SLU_N-	112 (3)	0.07 (0.01)	<0.001	0.86
GWS_N-	119 (5)	0.06 (0.01)	<0.001	0.84
BIO_N-	99 (5)	-0.08 (0.06)	0.22	0.18
FYM_N-	105 (7)	-0.02 (0.08)	0.85	0.00
FYMC_N-	107 (5)	0.07 (0.01)	0.27	0.04
CON_N-	114 (5)	0.06 (0.01)	<0.001	0.88
SLU_N+	118 (2)	0.00 (0.01)	0.30	0.15
GWS_N+	111 (3)	0.02 (0.01)	0.09	0.31
BIO_N+	82 (6)	-0.32 (0.16)	0.09	0.32
FYM_N+	119 (10)	-0.47 (0.22)	0.07	0.36
FYMC_N+	88 (5)	-0.16 (0.10)	0.15	0.24
CON_N+	104 (5)	0.33 (0.04)	<0.01	0.89

We found that after 9 applications of different types of OW, the Olsen-P stock dynamics due to the cumulative ΔP differed significantly across the different types of OW (Table 5-4). A ΔP of +100 kg P ha⁻¹ slightly raised the Olsen-P stock by 8 and 6 kg P ha⁻¹ (equivalent to +2 mg Olsen-P kg⁻¹) in the GWS_N- and SLU_N- treatments, respectively. For FYM, FYMC and BIO, Olsen-P decreased, although the cumulative ΔP slightly but constantly increased. For the OW in the N+ experiment, trends were still similar but more accentuated. Thus, Olsen-P stocks remained constant regardless of the positive values of the cumulative ΔP in the GWS_N+ and SLU_N+ treatments. These low efficiencies of OW to build reserves of Olsen-P in soils are likely explained by two processes. One was the precipitation of phosphate calcium minerals having sparingly decreased soluble P, reactions that could have occurred by modifying dissolution/precipitation equilibria by applying P in calcareous soils (Arvieu, 1980; Tunesi et al., 1999). The other was the presence of insoluble P forms in the applied OW, such as hydroxyapatite and inositol hexakisphosphate, which could have increased during the composting period (Hashimoto et al. 2014). However, it is worth noting that

composting has limited effects on the P dynamics. The N/P ratio of OW slightly decreased (possibly related to N losses during composting), with respectively 2.0, 1.7, 3.9 and 3.1 for SLU, GWS, FYM and FYMC, respectively. However, the slopes of the linear regressions between Olsen-P stocks and ΔP were similar between SLU and GWS, and between FYM and FYMC, respectively, both in N- and N+ experiment. This indicated a similar effect in the long term of P input from raw and composted OW.

Despite the decline in Olsen-P during the years of follow-up, there was no evidence to show that a decrease in Olsen-P led to losses in crop yields. Even in recent years of experimentation, the grain yields of maize (2017) and wheat (2018) did not significantly decrease in CON, BIO, FYM and FYMC compared with GWS and SLU, regardless of whether mineral N fertilizer was supplied. This might be explained by the agronomic threshold of soil Olsen-P: the crop yields will not respond to P applied when the soil Olsen-P remains over a critical value (Howard, 2006). According to a study carried out in southern France, the agronomic threshold of soil Olsen-P was 6.7 mg P kg⁻¹ for maize and 7.8 mg P kg⁻¹ for wheat on a deep alluvial silty-clay/clay soil with varying CaCO₃ (Colomb et al., 2007). Tang et al. (2009) also suggested that agronomic thresholds were 14.6 and 13.2 mg P kg⁻¹ for wheat and maize in calcareous soil, respectively. However, the Olsen-P concentrations in 2018 of our study ranged from 15 to 48 mg, which indicates that Olsen-P could not have been responsible primarily for limiting crop growth.

5.3.7 Exchangeable-K dynamics versus cumulative ΔK

The concentration of exchangeable K significantly decreased with time in SLU, GWS and CON for both the N- and N+ experiments, whereas it slightly increased in FYM_N- and FYMC_N- (Figure. S8). The cumulative ΔK varied greatly across treatments. In the N- experiment, the cumulative ΔK was -294, 555, 1540, 2284, 2215 and 27 kg ha⁻¹ for the SLU, GWS, BIO, FYM, FYMC and CON treatments, respectively. This ΔK increased after 2015 after the increase in the OW rate and digestate application in the N- experiment. In the N+ experiment, the cumulative ΔK varied from a minimum of -155 kg ha⁻¹ in CON to a maximum of 1628 kg ha⁻¹ in FYM.

Our regression slopes show that a ΔK of -100 kg K ha⁻¹ decrease exchangeable K by -117 kg K ha⁻¹ (equivalent 32 mg K kg⁻¹ soil) in the control treatment of N+ experiments (CON_N+) (Table 5-5). This value was relatively higher than that in previous studies. Madaras (2015) analyzed 6 sites with different soils and climates and found that -100 kg K ha⁻¹ offtake decreased exchangeable K by 4.5 mg K kg⁻¹. Correndo et al. (2021) reported that the removal of 100 kg K ha⁻¹ decreased exchangeable K (NH₄-OAc-K) by 24 kg K ha⁻¹ in a Mollisol. It is generally accepted that soil K depletion results from complex interactions, including soil mineralogy, soil clay content, biological processes and subsoil K supply (Correndo et al., 2021; Damar et al., 2020; Simonsson et al., 2007).

Table 5-5 Cumulative ΔK and associated variation in exchangeable K stock in the soil plowed layer after 18 years of experimentation.

Treatments	Exchangeable-K stock vs cumulative ΔK regression			
	Intercept	Slope	P-value	R ²
SLU_N-	1009 (33)	1.31 (0.16)	<0.001	0.89
GWS_N-	960 (37)	-0.60 (0.15)	0.004	0.66
BIO_N-	1032 (33)	-0.06 (0.04)	0.17	0.22
FYM_N-	1076 (57)	0.20 (0.05)	0.003	0.69
FYMC_N-	1042 (39)	0.21 (0.03)	<0.001	0.83
CON_N-	913 (56)	0.67 (0.34)	0.08	0.32
SLU_N+	958 (90)	2.20 (0.77)	0.02	0.50
GWS_N+	890 (38)	-0.81 (0.19)	0.002	0.69
BIO_N+	1035 (34)	-0.32 (0.06)	<0.001	0.81
FYM_N+	1140 (57)	0.02 (0.06)	0.70	0.02
FYMC_N+	984 (43)	0.05 (0.05)	0.35	0.11
CON_N+	885 (84)	1.17 (0.50)	0.05	0.41

Similar to the results of Olsen-P, the exchangeable-K dynamics due to the cumulative ΔK differed significantly across the types of OW (Figure 5-7). A ΔK of +100 kg K ha⁻¹ raised the exchangeable K stock by 20 and 21 kg K ha⁻¹ in the FYM_N- and FYMC_N- treatments, respectively. However, exchangeable K decreased with increasing cumulative ΔK in GWS and BIO. A ΔK of +100 kg K ha⁻¹ decreased the exchangeable K stock by 60 and 81 kg K ha⁻¹ in the BIO_N- and GWS_N- treatments, respectively. This discrepancy might be explained by the content of unexchangeable K in soil after different OW applications. FYM and FYMC, with the highest K input, raised both exchangeable and unexchangeable K contents in soil. It was reported that unexchangeable K levels in soil have a great impact on the relationship between exchangeable K and the ΔK . Exchangeable K increased with the positive ΔK in the soil with a high unexchangeable K content, whereas it decreased in the soil with a low unexchangeable K content (Madaras, 2015).

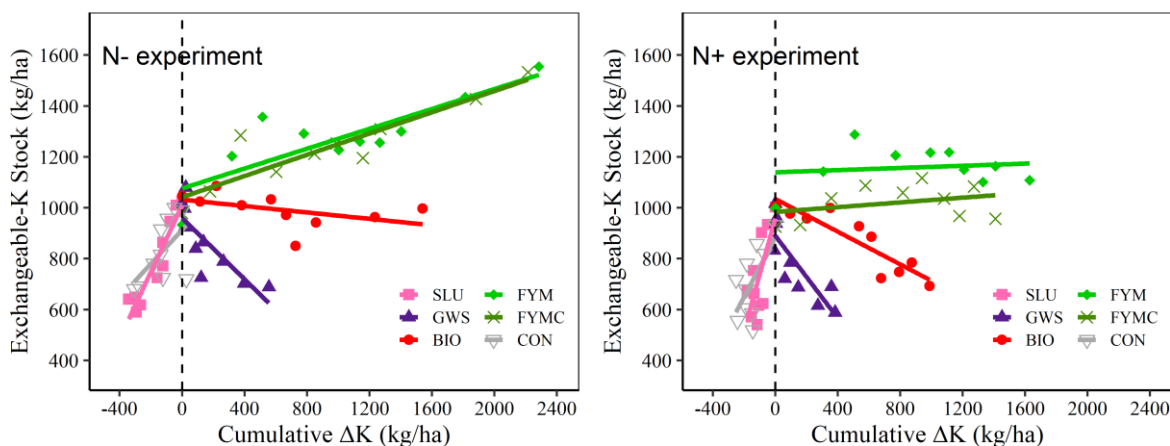


Figure 5-7 Relationship between cumulative ΔK and exchangeable-K stock in the soil plowed layer for the different CON and OW treatments in the N- and N+ experiments during the 18 years of experimentation (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control).

5.4 CONCLUSIONS

An 18-year field study confirmed that OW, except for SLU applied at usual farmer rates, significantly increased the SOC content, although the contribution of crop residues to the SOC stocks was higher. OW application increased crop yield compared with the unfertilized control. The application of OW to partially substitute mineral N fertilizer allowed crop yield to be sustained at the levels of mineral fertilizer only and saved mineral fertilizer. SLU saved the most N fertilizer, SLU and GWS saved the most P fertilizer, while FYM and FYMC saved the most K fertilizer. We also demonstrated that no negative impact on crop N, P, and K concentrations was associated with a partial substitution of mineral N with OW; digestate had even the potential to increase crop N concentrations, especially in wheat. Among all OW types, SLU and digestate had the greatest NFRVs, whereas BIO had the lowest NFRVs. The N-leaching potential did not increase with OW application in the long term. The relationship of soil available P and exchangeable K dynamics with the ΔP and ΔK , respectively, greatly depended on the kind of OW in this calcareous soil.

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Chapter VI

General discussion and perspectives

6 DISCUSSION AND PERSPECTIVES

6.1 MAIN RESULTS OF THE THESIS

6.1.1 General effect of OW applications on soil fertility and crop production

In both experiments studied in this work, the application of OW (except for SLU) increased SOC stocks, which is in line with previous studies with the same types of OW (Maillard and Angers, 2014; Paetsch et al., 2016). In QualiAgro, where a higher amount of OW was applied, soil active C as defined by Rock Eval pyrolysis increased after all types of OW application. However, the proportion of stable C in total soil organic C decreased in all OW treatments, suggesting that C inputs primarily accumulated in active soil C pools. However, crop residues contributed more to the increase in SOC than OW did, mainly in the Colmar site. The contributing to SOC storage in the long term differed among OW and between OW and crop residues, as suggested by various authors (Gerzabek et al., 1997; Levavasseur et al., 2020). The application of OW resulted in an increase in soil nitrogen, Olsen-P, and exchangeable K, although the extent of the effect varied depending on the specific type of OW used. For the soil with high pH (PROspective), application of SLU and GWS resulted in a slight decrease in soil pH, whereas for the site with lower pH (QualiAgro), all OW except for GWS led to a slight increase in soil pH. Recycling OW accompanied by an increase in soil TE availability. A previous study conducted at QualiAgro specifically investigating soil TE found that even after long-term application of OW, soil TE levels did not exceed calculated conservation thresholds, and there was no observed effect of repeated OW applications on TE contents in crop grains (Michaud et al., 2020). The increase in aggregate stability in our study was limited in our study, especially when compared to the study of Annabi et al. (2011), which was also conducted in QualiAgro. This highlights that the temporal variability of aggregate stability extends beyond its relationship with SOC.

Incorporation of OW into soil increased soil enzymatic activities, microbial biomass, but altered soil nematode communities by introducing potential different substrates. However, the addition of OW had a limited influence on the diversity of bacteria and fungi, which was consistent with the study by Bei et al. (2018). Overall, it appears that soil biological properties were less affected by OW compared to soil chemical properties. On the other hand, this also means that OW application (at least for the OW uses in these experiments) does not adversely affect soil biological activity, which is a common concern. Furthermore, we found that the TE introduced by OW did not negatively affect most of soil biological characteristics, possibly because the negative effects of TE were masked by the positive effect of OW application (Albiach et al., 2000).

The partial substitution of mineral nitrogen fertilizer with OW has been shown to maintain or even increase crop yields in the long term, which is consistent with the findings of many other studies (Gao et al., 2023; Zhang et al., 2020). However, full

substitution of mineral nitrogen fertilizer was insufficient to keep the crop yield, particularly in the second year after OW application. Over time, the positive effect of OW application on crop yield tends to increase in relation with the increase of soil organic matter stocks. For example, in QualiAgro, the combination of OW and low mineral N resulted in the same maize and wheat yields as the mineral N control after 3 and 6 applications of OW, respectively. Moreover, there was no significant influence of OW application on the harvest index (HI), which indicates that OW application did not impact the physiological efficiency of plants in converting to grain yield (Fosu-Mensah and Mensah, 2016). It is worth noting that full substitution of mineral nitrogen fertilizer with OW could decrease N concentrations in crop harvests compared to mineral fertilization, but there was no impact on P and K concentrations in crop harvests.

6.1.2 Importance of input fluxes to explain OW effects

OW input fluxes (C, N, P, K) and the potentially residual organic C applied in the OW, directly drive changes in soil physicochemical properties, and the cumulative application balance of these fluxes well explained the changes observed. We found a positive effect of realistic OW applications on SOC stocks. In particular, cumulative potentially residual organic C (PROC) input explained SOC variation better than cumulative C input. This confirms that PROC is a better indicator than total C for predicting the long-term storage of SOC, as suggested by Lashermes et al. (2009). For the GWS, BIO, FYM, FYMC, and MSW, the slow mineralization of organic N introduced by the OW resulted in an increase in soil N stocks after OW application. However, in the case of SLU and digestate, which have faster mineralization rates and higher mineral N content, there was no significant increase in soil N. The cumulative application balance also showed good correlations with soil N, P, and K. Trace elements introduced through OW remained incorporated into the soil, and we showed that the cumulative TE balance partly reflected variations in soil available TE content, particularly for Zn and Cu. These results were in line with a study by Michaud et al. (2020), which suggests that the positive TE mass balance is mainly due to the input flux of TE through OW.

OW has the potential to replace chemical fertilizers and sustain crop yields by supplying nutrients, but the magnitude of its effect depends on the quantity and characteristics of OW applied. Among many nutrients, the amount of N is a crucial factor that influences crop yield. The OW with higher mineral N content and N mineralization rate such as digestates provide readily available N for plant growth that is comparable to mineral fertilizers. For the OW with slow mineralisation rates, the cumulative soil total N increased after repeated OW application was the main driver of maize yield after repeated OW applications. Mineral N application from mineral fertilizers remained the most influential factor of wheat yield the year after OW application. It is well known that additional P and K application will not increase crop yields when soil Olsen-P and exchangeable K levels are above the agronomic critical value (Breker et al., 2019; Colomb et al., 2007). This finding is consistent with our own experiments, in which the

soil content of Olsen-P and exchangeable K exceeded the agronomic critical value, and as a result, neither the input of P and K nor their available contents in the soil significantly affected the relative crop yields.

Moreover, OW application increased soil microbe biomass and enzyme activities without adversely affecting soil biological activity. The impact of OW input on soil biology can vary depending on the input flux of C and nutrients (N, P and K). Although our GBM model in Chapter IV may not accurately predict changes in soil microbial communities, it has provided valuable information. Our analysis revealed that the balance of OW input fluxes (C, N, P and K) explained 74% of the variation in enzyme diversity, 33% of the variation in soil microbial biomass, and 28% of the variation in nematode diversity. Previous studies have indicated that the quantity and quality of organic inputs are the most significant factors affecting microbial biomass and community structure (Tu et al., 2006), and these changes are driven by elevated inputs of nutrients (Leff et al., 2015). In addition, the C balance was identified as the primary factor influencing the activity of urease and arylamidase, explaining 40% and 32% of the variation in activity, respectively. On the other hand, the N balance was identified as the primary factor influencing phosphatase activity, explaining 28% of the variation. This result is in line with the studies conducted by Jing et al. (2020), which identified soil inorganic N levels as the primary factor affecting phosphatase activity. Margalef et al. (2021) reported that the impact of N fertilization on phosphatase activity was greater than that of P fertilization, and the specific effects were influenced by the initial soil P level.

6.1.3 Influence on OW type of the observed effects

The effectiveness of OW application varies depending on the type of OW used. SLU and GWS were found to have the highest P content, leading to the highest soil Olsen-P stocks regardless of whether they were applied based on C (QualiAgro) or N (PROspective). However, SLU had a lower humification coefficient and was easily degradable, which resulted in no significant increase in soil C stocks. On the contrary, SLU had a higher ratio of mineral N, which allowed the maintenance of crop yields and reduction in chemical N fertilizer use. SLU and GWS also had relatively high TE content, particularly in Cu and Zn, leading to an increase in these elements in soil. FYM and FYMC had the highest K content, which is a common characteristic of dairy manure. These treatments resulted in the highest soil exchangeable K stocks and saved the most K fertilizer. However, FYM in PROspective resulted in decreased fungal richness, likely due to the introduction of dung and other saprotrophs that crowded out other fungal taxa (Sun et al., 2020). MSW had the lowest P and K content, resulting in the lowest improvement in available P and exchangeable K. Additionally, MSW had the lowest indicator of residual organic C (IROC) (Peltre et al., 2012), which resulted in the lowest increase in soil organic carbon (SOC) in QualiAgro. BIO had a relatively high IROC (70.1%) and was effective at increasing SOC after application. In the PROspective site, only BIO and GWS showed an increase in soil stable C as defined by Rock Eval pyrolysis,

suggesting that these OW had the most substantial impact on SOC accumulation. However, BIO had relatively low mineral N and P content, leading to a weaker increase in crop yield compared to other OW. Additionally, BIO had a negative impact on enzyme diversity in COL. Finally, digestate was characterized by higher ammonia content and had the potential to increase crop yield and N content in crop grain.

Composting can alter the properties of OW and, consequently, affect its effectiveness when applied as soil amendment. During composting, approximately 40% of the initial carbon is lost due to microbial metabolism (Ye et al., 2023), which can affect the total carbon (TC) content of the final product. In our study, we found that FYMC had a lower TC content than FYM, and GWS had a lower TC content than SLU (Appendix II Table S3). As labile carbon fractions are mineralized, the composted OW yields a more stable and humified organic matter (Hernández et al., 2006), resulting in higher IROC values for FYMC (67.8%) compared to FYM (57.0%) and for GWS (72.3%) compared to SLU (49.4%). In addition, TE present in OW became concentrated during composting due to weight loss from organic matter decomposition, CO₂ and water release, and mineralization processes (Liu et al., 2007). We observed that FYMC had a higher TE content than FYM, although the difference was not statistically significant. However, the concentrations of Cu and Zn in GWS were even lower than those of SLU. This could be attributed to the fact that GWS is a co-composting product of SLU and green waste, which dilutes the TE in SLU and thus reduces their concentration in the final product. Moreover, the effect of P input from raw and composted OW on soil Olsen-P dynamics were similar, whereas the dynamics of K were different between SLU and GWS. In addition, application of compost or raw OW may have different effects on soil biology. Our study revealed that both FYM and FYMC had a positive effect on plant-feeder nematodes in COL, but only FYM decreased PPI. This finding is consistent with the results of a previous study by Nahar et al. (2006), which suggested that raw manure may be more effective than composted manure in reducing plant parasitic nematodes.

6.1.4 Short-term versus long-term effects

In our experiments, the application of OW had different effects on crop yields in the short and long term. In the short term, crop yields decreased compared to conventional mineral fertilization treatments due to slow nutrient release in the applied OW (mainly composts or manure). In the long term, with the improvement of soil fertility, especially the increase of organic N content in the soil, the yield of crops increased over time. For example, in QualiAgro site, the combination of OW and low mineral N reached the same maize and wheat yield as the mineral N control after 3 and 6 applications of OW, respectively. For the same reasons, the nitrogen (N) fertilizer replacement value (NFRV) were low in the short term and increased over time (Gutser et al., 2005). For example, Schröder et al. (2007) indicated that the NFRV of cattle slurry was about 51–53% when applied in the first time and rises to 70% after 7–10 yearly applications. Thus, if NFRV of OW were estimated at short term, the requirements of OW or additional mineral N fertilization might be overestimated in the long term and might result in potential

environmental issues (e.g., N leaching). Similarly, the trace element content in the soil is not always easily detectable with a single application, but may accumulate to harmful levels in the cropland through repeated applications. We found that the trace element content increased with cumulative trace element balance over time. Therefore, long-term monitoring of trace elements in soil and their availability is necessary. Olsen-P and exchangeable K levels in soil can be affected by OW applications, and the long-term pattern of changes can vary depending on the type of OW and the soil conditions. Therefore, it is important to monitor available P and K levels in soil over time when using OW as a fertilizer to ensure that nutrient requirements of crops are being met and to avoid any potential negative environmental impacts from excessive nutrient application.

In contrast, microbial communities may change significantly in the short term and recover in the long term (Sadet-Bourgeteau et al., 2018). Previous studies have also emphasized that soil microbial biomass has a clear positive relationship with the accumulation of organic matter in the soil whereas the structure and diversity of microbial communities were unpredictable over time (Chernov and Zhelezova, 2020). This is because in the short term, the addition of organic matter can cause a priming effect, which stimulates the metabolic activity of microorganisms (Fontaine et al., 2003). However, the soil microbial community is usually resistant and resilient to exogenous microbes, most of the invasive microorganisms present in OW cannot survive in the long term in soil (Lourenço et al., 2018). We found that there were significant correlations between the microbial community and various soil physical and chemical properties. Thus, changes in the physical and chemical properties of the soil caused by OW input rather than organic matter inflow were the driving factors of the dynamics for the microbial community in the long term (Chernov and Zhelezova, 2020).

6.1.5 Other factors influencing the effects of OW

Soil characteristics

In addition to the effects of application rate and duration mentioned earlier, the different soil characteristics between the two sites may also be an important reason for the difference in observed effects. The soil in the QualiAgro study site is classified as a Luvisol, whereas the soil in the PROspective study site is a Calcosol, characterized by high levels of carbonate content. It is well known that carbonates in soil can contribute to the stabilization of soil organic matter by acting as a stabilizing agent and having protective effects on SOM mineralization (Clivot et al., 2017). As a result of the varying carbonate content, the rate of SOM decomposition was slower in PROspective than in QualiAgro. This may help to explain the findings from Chapter IV, where the ratio of stable C decreased compared to the control in all treatments with OW applied in QualiAgro, whereas this decrease only occurred in one treatment (FYM_N) in PROspective. Therefore, in comparison to previous research on P dynamics in QualiAgro conducted by Lauerjón et al. (2013), the P dynamics in PROspective are more complex

due to the significant impact of carbonate content.

Another finding in Chapter IV, where the application of OW in QualiAgro had a more pronounced effect on soil biology, microbial composition, and TE compared to PROspective, may also be attributed to the differences in soil characteristics at the two sites. Generally, due to the strong buffering ability of calcium carbonate, high SOM content and cation exchange capacity (CEC), calcareous soils have high pH buffering capacity compared to non-calcareous soils (Zhang et al., 2016). Research has demonstrated that SOM contributes to soil pH buffering capacity by acting as a pH buffer, mainly due to the presence of various functional groups such as amine carboxylic, alcohol, phenolic, and amide (Bashir et al., 2021). CEC can influence the soil pH buffering capacity by affecting the exchange of protons and base cations (Zhang et al., 2016). In our study, the initial SOM content (24.5 g/kg DM) and CEC (16.1 cmol/kg) in PROspective were much higher compared to QualiAgro (18.1 g/kg DM and 9.6 cmol/kg, respectively). As a result, the impact of OW on soil pH may be less noticeable in PROspective compared to QualiAgro, even if being applied in the same quality and quantity. As an example, in our study, we found that GWS, which is rich in phosphorus, significantly increased phosphatase activity in QUA but not in COL, and this difference may be attributed to the different SOM content between the two sites. A similar trend was reported by Adetunji et al. (2017), where phosphatase activity increased with P addition in soils with low organic matter content, but there were no changes observed in soils with high organic matter content.

Our correlation analysis results have already shown that soil pH is strongly related to various soil indices. Therefore, it is possible that the impact of OW application in PROspective may not be as significant as it is in QualiAgro. On the other hand, the high soil pH and processes of carbonate precipitation and sorption in calcareous soils result in a lower bioavailability of TE relative to their total content compared with non-calcareous soils (Zaragüeta et al., 2021). This may explain why the increase in trace element availability observed with the application of OW in the PROspective was less pronounced than in QualiAgro, in addition to the lower rates of OW application.

Climatic conditions

Another easily overlooked issue is the differing climatic conditions between QualiAgro and PROspective, which may also contribute to the differences in OW effects between the two sites. The rate of N mineralization is controlled by temperature, with mineralization rates increasing as temperature increases due to the chemical and enzymatic reactions which occur during decomposition are temperature dependent (Dessureault-Rompré et al., 2010). Thus, the N supply by OW may be affected accordingly. Although the average annual temperature is almost the same in QualiAgro and PROspective, average minimum temperature in PROspective were lower than QualiAgro. From December to March, the average minimum temperature in PROspective are 0°C, -2°C, -1°C and 2°C, respectively while the average minimum

temperature in QualiAgro were 2 degrees above during this period. Moreover, PROspective have more rainfall compared with QualiAgro especially in May and June.

Cropping system

In addition, the different crop rotations between QualiAgro and PROspective may also contribute to the differences between the two sites. The QualiAgro rotation was a 2-yr maize - wheat rotation until 2013, whereas PROspective was a 4-yr maize - wheat - sugarbeet - barley throughout the duration of the experiment. Previous studies have demonstrated the significant influence of crop rotation on N mineralization (Sharifi et al., 2008). The wheat grain yield and yield stability were found to be significantly higher with a 4-year rotation compared to a 2-year rotation (Cociu, 2012). These increase may be attributed to the increase of soil N availability (Spargo et al., 2011) and the decrease of weed seed banks, pests, and pathogens (Bennett et al., 2012). However, more diverse crop rotation is not necessarily always beneficial, soil health may decrease in the more diversified crop rotations (Agomoh et al., 2020). On the other hand, the two sites have different types of crops planted and different preceding crops when the soil samples were collected, which may impact soil microbial biomass and enzyme kinetics (Hamer et al., 2021).

Finally, unlike the OW applications in QualiAgro occurred in summer, OW applications in PROspective occurred in late autumn to early spring from 2001 to 2013, and switched to summer from 2014 onwards. Due to the mineralization of nitrogen in OW takes time, the different applications schedule result in different plant availability of N during a growing season. This might be an important factor that explain the different effect of OW on soil properties and crops yield between the sites.

6.2 BENEFITS AND LIMITATIONS OF MACHINE LEARNING IN SOIL SCIENCE

In our study, we used GBM to explore the driving factors of relative crop yields and the change of soil biological properties. Using machine learning regression methods can help reveal patterns and correlations in the data that might not be easily detected using traditional statistical methods, especially ideas that would otherwise go undetected (Wadoux et al., 2021). Thanks to the capability to capture non-linear relationships and interpretation of complex data, machine learning models successfully predict a wide range of outcomes related to soil, including soil microbial dynamics (Jha and Ahmad, 2018), crops yield (Meng et al., 2021), soil GHGs emission (Adjuik and Davis, 2022), soil aggregate stability (Rivera and Bonilla, 2020). Among the many machine learning algorithms available (e.g., Artificial Neural Network, Random Forest, GBM), we ultimately chose to use GBM in our study due to its high flexibility and the ability to customize it to our specific data-driven task (Natekin and Knoll, 2013). Furthermore, unlike Artificial Neural Networks that require all input data to be available for pre-processing and training, GBM can handle missing data without the need for pre-processing, making it a more flexible and efficient option for our study.

In data-driven GBM models, the researcher's knowledge and hypotheses often drive the selection of model inputs (Mount et al., 2020). Given that our paper aims to investigate the impacts of multiple applications of OW on farmland, we define the quantity of OW applied (incorporating C, N, P, and K fluxes) as the input to our model. Meanwhile, changes in yield and soil properties resulting from these applications are identified as the target outputs of the model. In Chapter III, we hypothesized that the nutrients introduced by OW and changes in soil properties resulting from OW application would lead to changes in relative crop yields. As our relative yield crop model utilizes the relative yield in different years as the target output, we include the soil properties prior to each OW application as additional model inputs, in addition to the amount of OW applied. By doing so, we aim to capture the effect of soil property changes that arise from repeated OW application in the model. Our hypothesis was confirmed by the results, as the 5-fold cross-validation R^2 values for GBM showed high predictive power for maize ($R^2=0.90$) and wheat ($R^2=0.93$), indicating that the selected input variables explained 90% and 93% of the variation in maize and wheat yield, respectively. In Chapter IV, we hypothesized that the nutrients introduced by OW would lead to changes in soil physicochemical and biological properties. In contrast to the model presented in Chapter III, which incorporated data from multiple years, we only measured soil properties in a single target year for this study. Since there were no notable variations in soil properties within the sites at the start of the experiment, we solely employed the cumulative application of OW as input to the crop model. However, the relatively low 5-fold cross-validation R^2 values for soil biological properties in the GBM model suggest that our hypothesis did not accurately reflect the changes in soil biological properties. It might be because of that our GBM model does not include some other potential driving factors, such as TE on soil biological properties. This decision was not based on our knowledge, but rather on the concern of overfitting due to the scale of the data we had. It is well known that model containing too many variables and little data may be overfitted and have poor generalization ability (van der Schaaf et al., 2012). Therefore, while data-driven machine learning models can be useful in identifying relationships in the data, it is important to carefully select and balance the inputs included in the model to ensure that the model is both accurate and generalizable.

Another concern with data-driven machine learning models is their lack of interpretability, which makes it difficult to understand the underlying causal relationships (Quaghebeur et al., 2022). Common machine learning models, such as GBM in our study, are considered black box models without considering how the mapping is achieved and therefore opaque to researcher (Krishnan, 2020). To open the black box and illustrate relationships, we use visualization methods called partial dependence plots (Friedman, 2001) to assist in model interpretability and assess the marginal effects of each covariate. These plots help us to assess the marginal effects of each covariate and gain a deeper understanding of how they impact the outcome. However, this method still has some limitations and deficiencies. The major limitations is that partial dependence plots assume that input variables are independent of one

another and may fail to capture heterogeneous effects (Ly et al., 2021). Additionally, partial dependence plots are typically represented in 2- or 3-dimensional formats because our ability to imagine more than 3 dimensions is limited (Petch et al., 2022). In Chapter III, we used 2-dimensional partial dependence plots to identify the relationship between relative crop yields and each driving factor, which mean all other factors not appearing on the diagram were set to their mean value in our study. As a result, these relationships can vary according to other factors change and may even show different trends. For example, we suggest that the soil pH range of 6.6 to 8.0 is beneficial to wheat yield, but this range may vary as other factors change. In addition, the interactions of other input factors with soil pH may cause partial dependence plots to inaccurately reflect the true relationship between soil pH and the yield response. Apart from using visualization methods, we also assess the importance of each variable to the target output by calculating their statistical contribution to the model in Chapters III and IV. The use of variable importance indicators based on inference forests is believed to strike a good balance between identifying significant variables and avoiding unnecessary flagging of correlated variables, according to Auret and Aldrich (2011). However, collinearity between variables can make it difficult to accurately assess the importance of individual variables in a model, as collinear variables within the model will share the importance score between them (Lucas, 2020). In the GBM model build in Chapter III, we observed a slight collinearity between mineral N input by OW and total N input by OW ($R^2=0.48$, $P<0.01$). This may result in reduced reliability in the assessment of variable importance in the model. In summary, although we employ techniques such as relative importance and partial dependence plots to interpret our models, they may not provide a complete understanding of the underlying mechanisms.

6.3 FUTURE PERSPECTIVES

Despite our analysis of the various impacts of OW on soil characteristics, many other impacts have not been covered in this study. These include the effects of OW on soil pathogens and drugs (Bourdat-Deschamps et al., 2017), greenhouse gas emissions (Zhejin Li et al., 2018), volatile organic compound emissions (Abis et al., 2018), groundwater contamination (Ananna et al., 2021), among others. Exploring these different impacts can help us gain a more comprehensive understanding of OW and maximize the benefits of expensive long-term experiments. Moreover, it will help us develop more informed and effective strategies for managing and conserving soil resources.

As was highlighted in the discussion, each experimental site presents its own unique set of conditions that can significantly impact the implementation of OW. To ensure the accurate transfer of experimental results into more widespread practical production settings, it is essential to attentively discriminate and analyze the spatial variability of the experimental results (such as temperature, precipitation, and soil characteristics) and the diversity of management strategies. To achieve this goal, we may need to

analyze a greater number of experimental sites for assessment. This is particularly efficient when combining the results from other sites within the experimental network like SOERE PRO network, as they may share commonalities that significantly reduce the difficulty of data analysis. In addition, modeling can be a powerful tool to extrapolate results from a particular study site to other conditions. However, as any model of real-world phenomena, soil or plant models must be calibrated and tested against empirical evidence (Batista et al., 2019), specific data from long-term experiments, to ensure their accuracy, reliability and applicability. Thus, the combination of LTE experiments and modeling can provide a more comprehensive understanding of the long-term effects of OW application on soil fertility and crop productivity, and inform the development of sustainable agricultural practices.

Consultation and collaboration with different participants and disciplines will aid in a more comprehensive understanding of the impacts of OW application. For example, we applied various composts including GWS, BIO, and FYMC at two sites to study their long-term impact on soil. However, as we know, various factors such as the moisture content of the raw materials, the ventilation rate of aerobic composts, and the duration of composting can have decisive effects on greenhouse gases during the composting process and on the final product (Yasmin et al., 2022). If future experiments can link the composting process and long-term application effects, it will provide us with more detailed references. For instance, the impact of the composting process on compost properties and the resulting effects on soil properties could be explored. The effect of the composting process on the overall greenhouse gas emissions could also be studied during the life cycle of agricultural production (emissions during composting process, during compost storage, and after land application). Admittedly, life cycle assessment (LCA) may be an efficient tool in this regard, as it can help comprehensive evaluation of the environmental impact of a product, process, or service over its entire life cycle, from raw material extraction to disposal. Nevertheless, field experiments remain an indispensable source of evidence to document LCA and support informed decision-making.

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Appendix I

Substitution of mineral N fertilizers with organic wastes in two long term field experiments: dynamics and drivers of crop yields

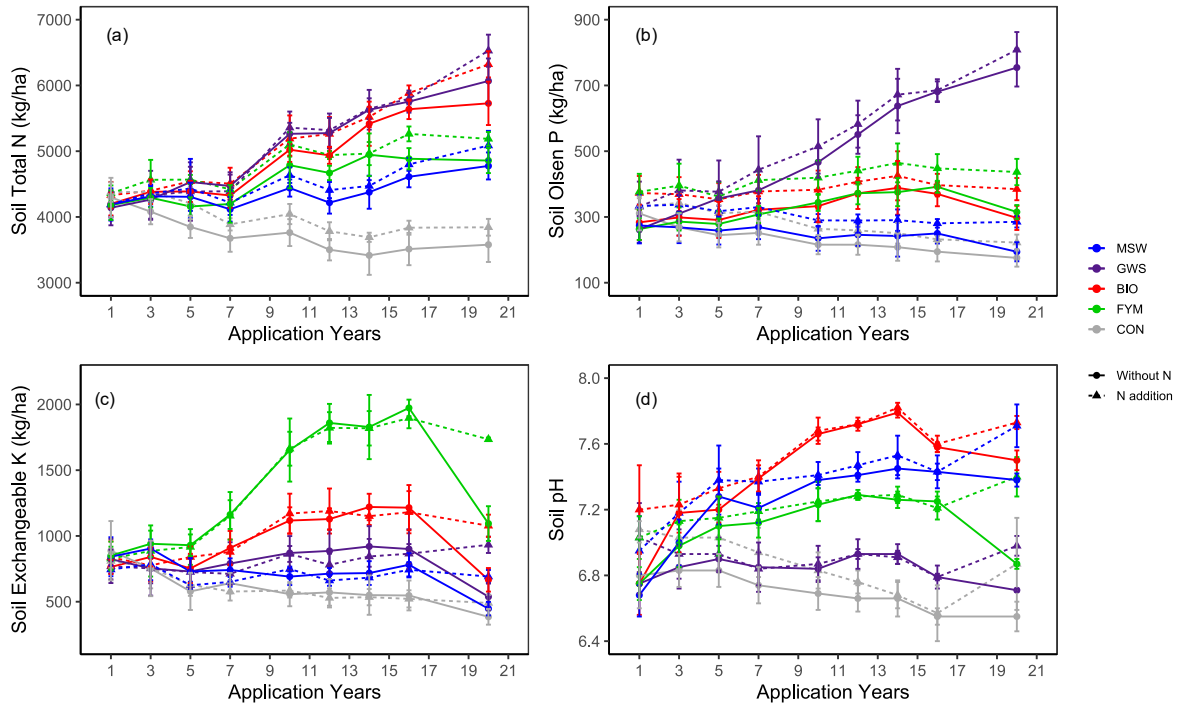


Figure.S1 Dynamics of (a) soil N stocks, (b) soil Olsen-P stocks, (c) soil exchangeable K stocks and (d) soil pH in the plowed layer under the different OW applications and CON in QUA. Error bars are the standard deviations (four replicates).

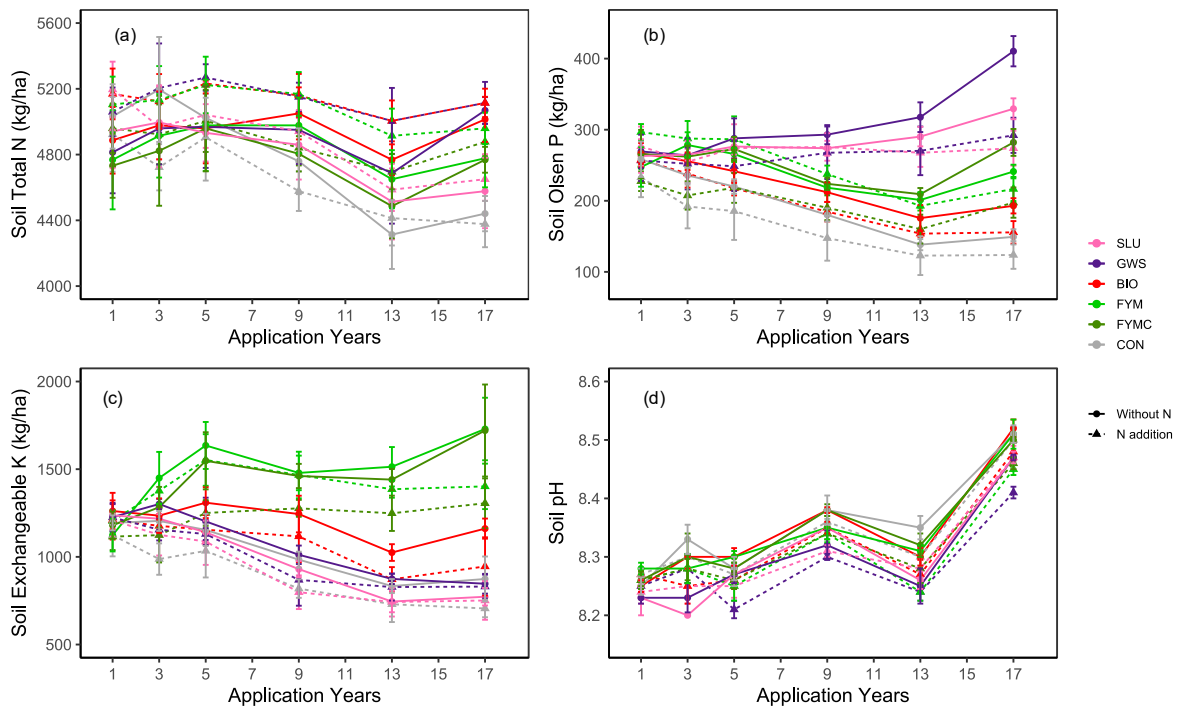


Figure.S2 Dynamics of (a) soil N stocks, (b) soil Olsen-P stocks, (c) soil exchangeable K stocks and (d) soil pH in the plowed layer under the different OW applications and CON in COL. Error bars are the standard deviations (four replicates).

Table S.1: Amount of organic wastes (OW) applied in maize - wheat succession over the period 2001-2018 in QUA (mean \pm sd).

OW	Applica tion period (year. month)	Quantity (t ha⁻¹ FM)	Organic C (kg ha⁻¹)	Total N (kg ha⁻¹)	Mineral N (kg ha⁻¹)	P₂O₅ (kg ha⁻¹)	K₂O (kg ha⁻¹)
GWS	1998.9	20.0	2919 \pm 119	303 \pm 7	34 \pm 1	202 \pm 11	226 \pm 2
GWS	2000.9	33.0	3786 \pm 70	366 \pm 8	66 \pm 2	323 \pm 3	575 \pm 10
GWS	2002.9	34.8	6454 \pm 872	428 \pm 25	66 \pm 3	500 \pm 187	201 \pm 55
GWS	2004.9	27.4	5359 \pm 378	362 \pm 8	71 \pm 2	546 \pm 55	212 \pm 18
GWS	2007.9	26.7	3767 \pm 60	369 \pm 12	63 \pm 3	552 \pm 8	264 \pm 3
GWS	2011.9	19.3	3697 \pm 241	349 \pm 10	78 \pm 2	556 \pm 40	187 \pm 7
GWS	2013.9	25.9	4478 \pm 44	392 \pm 8	112 \pm 2	481 \pm 14	359 \pm 10
GWS	2015.9	11.9	2220 \pm 46	179 \pm 3	35 \pm 5	225 \pm 0	144 \pm 2
GWS	2017.9	13.7	2192 \pm 25	185 \pm 4	51 \pm 2	291 \pm 14	126 \pm 2
BIO	1998.9	21.0	2557 \pm 215	265 \pm 15	20 \pm 0	114 \pm 3	310 \pm 5
BIO	2000.9	40.3	4314 \pm 184	292 \pm 12	5 \pm 1	218 \pm 49	524 \pm 11
BIO	2002.9	37.2	4586 \pm 187	358 \pm 8	20 \pm 2	223 \pm 9	663 \pm 11
BIO	2004.9	30.3	3311 \pm 39	258 \pm 10	14 \pm 1	174 \pm 22	557 \pm 14
BIO	2007.9	25.2	3737 \pm 82	368 \pm 3	54 \pm 1	330 \pm 11	383 \pm 7
BIO	2011.9	15.2	3630 \pm 123	293 \pm 2	20 \pm 3	145 \pm 4	319 \pm 2
BIO	2013.9	25.9	4587 \pm 89	344 \pm 1	42 \pm 1	170 \pm 2	556 \pm 8
BIO	2015.9	10.0	2196 \pm 48	169 \pm 5	13 \pm 0	80 \pm 2	204 \pm 10
BIO	2017.9	9.9	2431 \pm 48	200 \pm 2	13 \pm 2	108 \pm 5	221 \pm 2
MSW	1998.9	14.0	3039 \pm 177	202 \pm 3	22 \pm 0	90 \pm 5	131 \pm 4
MSW	2000.9	23.6	6002 \pm 65	396 \pm 12	31 \pm 1	158 \pm 4	226 \pm 3
MSW	2002.9	12.7	2624 \pm 164	156 \pm 9	22 \pm 1	61 \pm 3	97 \pm 2
MSW	2004.9	17.5	3850 \pm 126	246 \pm 18	19 \pm 1	107 \pm 3	162 \pm 1
MSW	2007.9	23.1	3773 \pm 68	158 \pm 7	74 \pm 1	77 \pm 6	95 \pm 3
MSW	2011.9	21.1	3264 \pm 175	227 \pm 1	31 \pm 1	153 \pm 2	247 \pm 6
MSW	2013.9	21.1	4327 \pm 63	212 \pm 2	50 \pm 0	119 \pm 2	161 \pm 1
MSW	2015.9	8.3	2089 \pm 32	98 \pm 0	10 \pm 0	64 \pm 1	79 \pm 0
MSW	2017.9	10.4	2835 \pm 41	124 \pm 2	17 \pm 10	64 \pm 1	92 \pm 1
FYM	1998.9	55.0	3756 \pm 69	314 \pm 4	76 \pm 11	187 \pm 7	544 \pm 14
FYM	2000.9	25.2	4242 \pm 362	214 \pm 17	17 \pm 7	90 \pm 10	414 \pm 17
FYM	2002.9	41.3	5400 \pm 294	420 \pm 14	26 \pm 1	250 \pm 24	681 \pm 16
FYM	2004.9	36.6	5268 \pm 214	320 \pm 7	21 \pm 1	137 \pm 7	658 \pm 16
FYM	2007.9	37.4	3662 \pm 56	277 \pm 8	19 \pm 0	189 \pm 9	544 \pm 24
FYM	2011.9	15.8	3738 \pm 23	209 \pm 5	5 \pm 0	118 \pm 2	397 \pm 16
FYM	2013.9	33.3	2902 \pm 77	229 \pm 4	14 \pm 5	172 \pm 9	612 \pm 20
FYM	2015.9	28.4	3416 \pm 131	190 \pm 6	5 \pm 0	152 \pm 3	375 \pm 6
FYM	2017.9	13.4	2054 \pm 59	131 \pm 14	3 \pm 2	78 \pm 8	291 \pm 17

Table S.2: Amount of organic wastes (OW) applied in maize - wheat succession over the period 2001-2013 in COL_N+ and in COL_N- treatments (mean \pm sd).

OW	Applica tion period (year.m onth)	Quantity (t ha⁻¹ FM)	Organic C (kg ha⁻¹)	Total N (kg ha⁻¹)	Mineral N (kg ha⁻¹)	P₂O₅ (kg ha⁻¹)	K₂O (kg ha⁻¹)
SLU	2001.5	18.7	1098	201	36	242	26
SLU	2003.2	16.4	1077 \pm 20	185 \pm 2	19 \pm 6	211 \pm 8	15 \pm 0
SLU	2005.1	14.2	1003 \pm 9	153 \pm 1	57 \pm 4	177 \pm 5	16 \pm 0
SLU	2009.2	13.8	1021 \pm 120	16 \pm 20	53 \pm 1	156 \pm 19	26 \pm 3
SLU	2013.2	12.9	847 \pm 14	150 \pm 3	37 \pm 2	138 \pm 3	16 \pm 1
GWS	2001.5	21.0	3744	263	27	237	95
GWS	2003.2	12.5	2443 \pm 21	141 \pm 1	25 \pm 2	201 \pm 3	52 \pm 1
GWS	2005.1	16.8	2084 \pm 82	164 \pm 12	38 \pm 4	230 \pm 19	68 \pm 6
GWS	2009.2	15.3	1524 \pm 109	172 \pm 7	27 \pm 1	213 \pm 6	121 \pm 4
GWS	2013.2	11.2	1995 \pm 47	180 \pm 4	20 \pm 3	167 \pm 7	225 \pm 4
BIO	2001.5	11.1	2407	158	3	81	201 \pm 3
BIO	2003.2	10.7	2165 \pm 218	166 \pm 12	2 \pm 1	83 \pm 3	187 \pm 5
BIO	2005.1	16.6	2151 \pm 55	159 \pm 4	4 \pm 1	87 \pm 14	256 \pm 6
BIO	2009.2	14.6	1386 \pm 29	128 \pm 4	8 \pm 1	62 \pm 3	173 \pm 5
BIO	2013.2	18.8	2101 \pm 30	173 \pm 5	8 \pm 1	82 \pm 3	208 \pm 4
FYM	2001.5	19.4	3188	301	32	166	453
FYM	2003.2	19.1	2900 \pm 215	173 \pm 17	5 \pm 1	99 \pm 13	301 \pm 37
FYM	2005.1	14.9	3170 \pm 75	215 \pm 14	14 \pm 3	89 \pm 5	391 \pm 11
FYM	2009.2	9.3	2877 \pm 121	163 \pm 1	6 \pm 1	81 \pm 1	226 \pm 14
FYM	2013.2	32.5	2219 \pm 34	140 \pm 6	12 \pm 5	87 \pm 9	221 \pm 16
FYMC	2001.5	22.3	2020	142	3	118	281
FYMC	2003.2	25.2	2408 \pm 65	164 \pm 3	2 \pm 0	108 \pm 5	299 \pm 4
FYMC	2005.1	41.6	2780 \pm 29	188 \pm 0	6 \pm 2	91 \pm 2	343 \pm 5
FYMC	2009.2	41.8	1888 \pm 16	141 \pm 4	2 \pm 1	79 \pm 2	231 \pm 5
FYMC	2013.2	36.9	1980 \pm 35	133 \pm 2	3 \pm 1	94 \pm 2	190 \pm 13

Table S.3: Amount of organic wastes (OW) applied in maize - wheat succession over the period 2014-2018 in the in COL_{N+} and in COL_{N-} treatments (mean ± sd).

OW	Applica tion period (year.m onth)	Quantity (t ha ⁻¹ FM)	Organic C (kg ha ⁻¹ DM)	Total N (kg ha ⁻¹ DM)	Mineral N (kg ha ⁻¹ DM)	P ₂ O ₅ (kg ha ⁻¹ DM)	K ₂ O (kg ha ⁻¹ DM)
SLU _{N-}	2014.7	21.0	1576 ± 276	231 ± 46	142 ± 8	334 ± 6	40 ± 1
SLU _{N-}	2016.7	12.5	878 ± 7	137 ± 2	64 ± 3	146 ± 4	19 ± 0
GWS _{N-}	2014.7	16.8	5601 ± 435	676 ± 88	116 ± 17	931 ± 51	336 ± 2
GWS _{N-}	2016.7	22.9	2372 ± 123	242 ± 10	82 ± 6	504 ± 22	153 ± 5
BIO _{N-}	2014.7	15.3	6715 ± 246	564 ± 127	52 ± 1	336 ± 5	625 ± 4
BIO _{N-}	2016.7	11.6	3229 ± 112	244 ± 3	53 ± 1	168 ± 3	329 ± 12
FYM _{N-}	2014.7	11.2	6613 ± 1103	350 ± 38	114 ± 3	221 ± 50	681 ± 83
FYM _{N-}	2016.7	11.1	4695 ± 331	179 ± 17	31 ± 5	122 ± 17	532 ± 28
FYMC _{N-}	2014.7	10.7	4196 ± 119	286 ± 82	11 ± 0	280 ± 43	844 ± 43
FYMC _{N-}	2016.7	16.6	1891 ± 52	157 ± 11	2 ± 0	150 ± 12	369 ± 19
SLU _{N+}	2014.7	11.9	891 ± 156	131 ± 26	80 ± 8	189 ± 3	22 ± 1
SLU _{N+}	2016.7	13.0	915 ± 7	143 ± 2	67 ± 3	153 ± 4	20 ± 0
GWS _{N+}	2014.7	11.9	1750 ± 136	211 ± 27	36 ± 17	291 ± 16	105 ± 1
GWS _{N+}	2016.7	22.9	1484 ± 77	152 ± 6	52 ± 6	316 ± 14	96 ± 3
BIO _{N+}	2014.7	15.3	2243 ± 82	188 ± 42	17 ± 1	112 ± 2	209 ± 1
BIO _{N+}	2016.7	11.6	2021 ± 72	153 ± 2	33 ± 1	105 ± 2	206 ± 7
FYM _{N+}	2014.7	11.2	2492 ± 416	132 ± 14	43 ± 3	83 ± 19	257 ± 31
FYM _{N+}	2016.7	11.1	2938 ± 207	112 ± 10	19 ± 5	76 ± 11	333 ± 17
FYMC _{N+}	2014.7	10.7	1431 ± 41	97 ± 28	4 ± 0	95 ± 15	288 ± 15
FYMC _{N+}	2016.7	16.6	1184 ± 33	98 ± 7	2 ± 0	94 ± 7	231 ± 12

Table S.4: Amount of digestate applied in maize - wheat succession over the period 2014-2018 in the in COL (mean ± sd).

Treatments	Applica tion period (year.m onth)	Quantity (t ha ⁻¹ FM)	Organic C (kg ha ⁻¹ DM)	Total N (kg ha ⁻¹ DM)	Mineral N (kg ha ⁻¹ DM)	P ₂ O ₅ (kg ha ⁻¹ DM)	K ₂ O (kg ha ⁻¹ DM)
SLU _{N-}	2016.3	16.0	408 ± 9	140 ± 4	60 ± 9	41 ± 1	77 ± 1
SLU _{N-}	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
GWS _{N-}	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
BIO _{N-}	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
FYM _{N-}	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
FYMC _{N-}	2016.3	16.0	408 ± 9	140 ± 4	60 ± 9	41 ± 1	77 ± 1
FYMC _{N-}	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
CON _{N-}	2015.3	60.0	1348 ± 11	436 ± 22	226 ± 33	88 ± 5	309 ± 11
CON _{N-}	2016.3	26.7	681 ± 15	234 ± 6	99 ± 15	69 ± 2	129 ± 2
CON _{N-}	2017.5	26.0	783 ± 15	229 ± 28	91 ± 13	67 ± 1	132 ± 6
CON _{N-}	2018.4	29.5	715 ± 18	176 ± 5	142 ± 3	70 ± 3	124 ± 3

Appendix II

Changes, interactions and drivers of soil chemical, physical and biological properties after repeated application of organic wastes in two contrasted long term field experiments in France

Table S1: Amount of cumulative applied organic waste (OW) over the 2001-2018 period in site COL.

OW	Quantity (t/ha DM)	Organic C (t/ha)	Total N (kg/ha)	Mineral N (kg/ha)	P₂O₅ (kg/ha)	K (kg/ha)
SLU_N-	25.5	9.2	1493	449	1724	160
GWS_N-	77.9	23.1	2164	353	2964	1058
BIO_N-	93.4	24.0	1936	97	1076	2016
FYM_N-	76.1	30.3	1823	215	1069	2843
FYMC_N-	60.3	21.1	1523	33	1155	2676
SLU_N+	23.6	8.6	1398	388	1585	147
GWS_N+	62.2	18.4	1609	260	2135	819
BIO_N+	73.5	18.3	1469	62	789	1569
FYM_N+	61.6	24.4	1538	143	886	2325
FYMC_N+	50.0	17.6	1276	25	914	2099

Table S2: Amount of cumulative applied digestate over the 2014-2018 period in site COL.

OW	Quantity (t/ha DM)	Organic C (t/ha)	Total N (kg/ha)	Mineral N (kg/ha)	P₂O₅ (kg/ha)	K (kg/ha)
SLU_N-	1.2	0.4	140	60	41	64
FYM_N-	1.2	0.4	140	60	41	64
CON_N-	7.3	2.8	930	270	223	473

Table S3. Mean characteristics \pm standard deviations of organic waste applied 9 times in the COL experiment from 2001 to 2018. For digestate, there were 4 applications from 2015 to 2018. For each year, 3 samples were analyzed for each OW. Values with the same lowercase letters in a column are not significantly different at $P < 0.05$. Different letters indicate statistically significant differences at a P -value of 0.05 (Duncan test).

OW	Organic C g/kg DM	Total N g/kg DM	P₂O₅ g/kg DM	K g/kg DM	Cd mg/kg DM	Cr mg/kg DM	Cu mg/kg DM	Ni mg/kg DM	Pb mg/kg DM	Zn mg/kg DM
SLU	370 \pm 29 ab	60 \pm 5 b	67 \pm 4 a	6 \pm 1 f	1.0 \pm 0.2 a	39 \pm 7 a	325 \pm 80 a	31 \pm 7 a	51 \pm 16 b	630 \pm 160 a
GWS	291 \pm 46 c	27 \pm 5 c	38 \pm 13 b	14 \pm 5 e	0.8 \pm 0.2 ab	44 \pm 8 a	196 \pm 55 b	28 \pm 6 a	50 \pm 15 b	453 \pm 115 b
BIO	258 \pm 30 d	20 \pm 3 c	11 \pm 2 d	21 \pm 4 d	0.6 \pm 0.4 c	45 \pm 22 a	90 \pm 45 c	19 \pm 8 b	107 \pm 125 a	257 \pm 91 c
FYM	399 \pm 18 a	24 \pm 5 c	14 \pm 4 cd	37 \pm 6 c	0.3 \pm 0.2 c	6 \pm 3 c	30 \pm 9 d	4 \pm 1 c	2 \pm 0 b	153 \pm 60 c
FYMC	348 \pm 29 b	26 \pm 2 c	19 \pm 4 c	43 \pm 8 b	0.4 \pm 0.1 c	11 \pm 4 c	42 \pm 16 cd	5 \pm 2 c	4 \pm 1 b	210 \pm 90 c
Digestate	378 \pm 28 ab	114 \pm 18 a	33 \pm 4 b	60 \pm 13 a	0.1 \pm 0.0 c	24 \pm 3 b	69 \pm 25 cd	14 \pm 1 b	5 \pm 1 b	231 \pm 56 c

Table S4: Amount of cumulative applied organic waste (OW) over the 1998-2018 period in site QUA.

OW	Quantity (t/ha DM)	Organic C (t/ha)	Total N (kg/ha)	Mineral N (kg/ha)	P₂O₅ (kg/ha)	K (kg/ha)
GWS_LEG	148.5	39.2	3420	638	4373	2204
BIO_LEG	172.2	35.3	2883	215	1795	3660
MSW_LEG	109.7	33.7	1931	291	899	1139
FYM_LEG	115.1	36.5	2510	202	1500	4080
GWS_N	164.8	43.6	3784	724	4889	2428
BIO_N	187.5	39.9	3252	240	1983	4012
MSW_N	124.5	38.6	2153	318	1026	1281
FYM_N	127.3	40.7	2777	207	1678	4556

Table S5. Mean characteristics \pm standard deviations of organic waste applied 11 times in the QUA experiment from 1998 to 2017. For each year, 3 samples were analyzed for each OW. Values with the same lowercase letters in a column are not significantly different at $P < 0.05$. Different letters indicate statistically significant differences at a P -value of 0.05 (Duncan test).

OW	Organic C g/kg DM	Total N g/kg DM	P₂O₅ g/kg DM	K g/kg DM	Cd mg/kg DM	Cr mg/kg DM	Cu mg/kg DM	Ni mg/kg DM	Pb mg/kg DM	Zn mg/kg DM
GWS	266 \pm 40 b	23 \pm 2 a	30 \pm 7 a	15 \pm 4 c	1.0 \pm 0.6 ab	40 \pm 7 b	176 \pm 39 a	26 \pm 7 a	60 \pm 11 b	425 \pm 95 a
BIO	227 \pm 56 b	19 \pm 5 b	11 \pm 4 bc	22 \pm 3 b	0.6 \pm 0.6 b	35 \pm 15 b	60 \pm 23 b	22 \pm 23 ab	77 \pm 55 ab	223 \pm 94 b
MSW	314 \pm 41 a	17 \pm 2 b	8 \pm 2 c	10 \pm 3 d	1.4 \pm 0.6 a	81 \pm 52 a	148 \pm 78 a	30 \pm 16 a	134 \pm 80 a	430 \pm 215 a
FYM	327 \pm 62 a	22 \pm 3 a	13 \pm 2 b	36 \pm 3 a	1.0 \pm 1.0 ab	31 \pm 22 b	91 \pm 63 b	11 \pm 8 b	101 \pm 118 ab	331 \pm 172 ab

Table S6. The cumulative C, N, P and K input and export in COL.

OW	C (t/ha)		N (kg/ha)		P ₂ O ₅ (kg/ha)		K (kg/ha)	
	OW input	Crop input	input	export	input	export	input	export
SLU_N+	9	69±4	2275	1818±47	1690	856±10	662	749±13
GWS_N+	18	74±4	3201	1865±48	2240	855±15	1178	764±21
BIO_N+	18	65±3	2973	1803±29	1021	811±12	1770	751±16
FYM_N+	24	70±2	2952	1844±77	1074	860±24	2471	810±43
FYMC_N+	18	68±2	2846	1897±44	1116	853±28	2245	806±25
CON_N+	0	70±2	1908	1990±47	521	773±19	601	726±17
SLU_N-	10	67±3	1633	1503±77	1870	799±31	370	725±21
GWS_N-	23	64±2	2164	1321±25	3069	750±10	1203	711±17
BIO_N-	24	49±3	1936	1229±60	1181	698±33	2161	684±30
FYM_N-	30	57±3	1823	1378±54	1174	783±26	2988	765±27
FYMC_N-	21	55±3	1664	1320±59	1301	751±28	2885	730±28
CON_N-	3	48±4	899	1176±41	328	610±31	618	663±19

Note: Due to some data of C/N/P/K in crop grain/residues of were missing, the estimated input/export for the missing year was calculated by the amount of crop yield/residues multiply by the mean content of the corresponding plots of other years.

Table S7. The cumulative C, N, P and K input and export in QUA.

OW	C (t/ha)		N (kg/ha)		P ₂ O ₅ (kg/ha)		K (kg/ha)	
	OW input	Crop input	input	export	input	export	input	export
GWS_N	44	37±1	5486	2582±94	4925	2663±136	2440	1094±61
BIO_N	40	36±1	4807	2404±71	2019	2594±81	4025	1043±46
MSW_N	39	34±2	3896	2429±94	1090	2489±78	1304	1018±49
FYM_N	41	37±2	4326	2517±51	1743	2711±56	4579	1139±26
CON_N	2	32±1	2038	2035±53	217	2207±76	73	858±38
GWS_LEG	39	29±2	4164	1891±83	4373	2133±90	2204	816±38
BIO_LEG	35	29±1	3507	1628±48	1795	2003±50	3660	726±20
MSW_LEG	34	27±1	2662	1697±52	899	2003±72	1139	755±25
FYM_LEG	37	29±1	3117	1719±97	1500	2058±31	4080	787±19
CON_LEG	0	20±1	488	1058±74	0	1420±88	0	533±35

Note: Due to some data of C/N/P/K in crop grain/residues of were missing, the estimated input/export for the missing year was calculated by the amount of crop yield/residues multiply by the mean content of the corresponding plots of other years. Moreover, for the QUA_LEG treatment, the flux export and C input from alfalfa were not considered since the data were missing.

Table S8. The cumulative trace elements input by OW and export by crop grain in COL.

OW	Zn (g/ha)		Pb (g/ha)		Ni (g/ha)		Cu (g/ha)		Cd (g/ha)		Cr (g/ha)	
	input	export	input	export	input	export	input	export	input	export	input	export
SLU_N+	14464	2834	1241	25	743	35	7338	513	25	2	901	29
GWS_N+	26945	2866	3185	21	1719	27	11272	517	49	2	2693	25
BIO_N+	20418	2892	8734	24	1482	25	7118	507	53	2	3555	30
FYM_N+	9088	2820	123	21	220	31	1825	510	19	3	363	30
FYMC_N+	9451	2965	179	24	248	26	1933	524	19	2	480	28
CON_N+	0	2896	0	25	0	31	0	504	0	2	0	24
SLU_N-	16951	2566	1412	23	846	33	8388	433	27	2	1075	28
GWS_N-	34670	2477	3813	20	2189	25	15292	412	62	2	3469	24
BIO_N-	26051	2407	11537	22	1884	23	9608	355	69	2	4360	29
FYM_N-	11736	2539	152	20	281	30	2391	394	22	3	465	30
FYMC_N-	13402	2480	225	24	341	25	2728	381	24	2	664	28
CON_N-	2159	2250	47	23	127	26	689	352	1	2	224	23

Note: Due to some data of trace elements content in crop grain of COL_N- experiment were missing, the estimated export for the missing year was calculated by the yield multiply by the trace elements content in corresponding OW treatment in COL_N+ experiment.

Table S9. The cumulative trace elements input by OW and export by crop grain in QUA.

OW	Zn (g/ha)		Pb (g/ha)		Ni (g/ha)		Cu (g/ha)		Cd (g/ha)		Cr (g/ha)	
	input	export	input	export	input	export	input	export	input	export	input	export
GWS_N	59602	3051	9117	22	4012	41	25557	457	150	3	5931	44
BIO_N	42298	3085	15926	22	4769	34	10871	479	126	3	6826	46
MSW_N	45327	3191	15470	24	3356	42	15955	495	154	3	9497	48
FYM_N	40630	3207	13917	24	1469	41	11303	488	133	3	4115	50
CON_N	0	2642	0	24	0	40	0	420	0	3	0	43
GWS_LEG	59602	2642	9117	18	4012	31	25557	413	150	2	5931	34
BIO_LEG	42298	2522	15926	15	4769	29	10871	373	126	2	6826	34
MSW_LEG	45327	2625	15470	18	3356	35	15955	393	154	2	9497	34
FYM_LEG	40630	2528	13917	17	1469	34	11303	394	133	2	4115	35
CON_LEG	0	1818	0	15	0	29	0	284	0	2	0	27

Note: Due to some data of trace elements content in crop grain/residues of QUA_LEG experiment were missing, the estimated export for the missing year was calculated by the crop yield/residues multiply by the trace elements content in corresponding OW treatment in QUA_N experiment. The flux export from alfalfa was not considered since the data were missing.

Table S10: Mean characteristics \pm standard deviations of soil properties in the control treatments of QUA and COL in 2018. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$. Different letters indicate statistically significant differences at a P -value of 0.05 (Duncan test). Name of abbreviations can be found in the abbreviation table.

Soil property	QUA_N	QUA_LEG	COL_N-	COL_N+
Soil physico-chemistry				
TC (g/kg dry soil)	10.1 \pm 0.4 b	9.0 \pm 0.5 c	13.8 \pm 0.5 a	13.2 \pm 0.3 a
TN (g/kg dry soil)	0.99 \pm 0.02 b	0.93 \pm 0.06 b	1.29 \pm 0.04 a	1.24 \pm 0.03 a
Soil.C/N	10.2 \pm 0.4 a	9.7 \pm 0.1 b	10.7 \pm 0.1 a	10.7 \pm 0.3 a
Soil.pH (water)	6.87 \pm 0.25 b	6.55 \pm 0.08 c	8.49 \pm 0.03 a	8.50 \pm 0.02 a
Soil.CEC (cmol+/kg)	9.35 \pm 0.50 b	8.96 \pm 0.45 b	16.70 \pm 0.21 a	17.05 \pm 0.27 a
EK (cmol+/kg)	0.31 \pm 0.04 b	0.21 \pm 0.03 c	0.51 \pm 0.01 a	0.36 \pm 0.02 b
AP (P ₂ O ₅ g/ kg dry soil)	0.05 \pm 0.01 b	0.06 \pm 0.01 a	0.05 \pm 0.00 b	0.03 \pm 0.01 c
MWD.FW (mm)	0.28 \pm 0.08 c	0.86 \pm 0.32 b	1.46 \pm 0.09 a	0.62 \pm 0.09 b
MWD.WS (mm)	0.31 \pm 0.05 b	0.57 \pm 0.20 ab	0.50 \pm 0.02 ab	0.70 \pm 0.26 a
MWD.MB (mm)	0.84 \pm 0.08 c	0.87 \pm 0.07 c	1.49 \pm 0.10 a	1.26 \pm 0.07 b
Active.C (g/ kg dry soil)	3.35 \pm 0.10 ab	2.90 \pm 0.35 b	3.65 \pm 0.25 a	3.72 \pm 0.39 a
Stable.C (g/ kg dry soil)	5.77 \pm 0.33 b	5.40 \pm 0.20 b	6.85 \pm 0.34 a	6.71 \pm 0.20 a
SC.ratio	0.63 \pm 0.02 a	0.65 \pm 0.03 a	0.65 \pm 0.02 a	0.64 \pm 0.02 a
Extractable trace metal				
Cr (mg/ kg dry soil)	0.07 \pm 0.02 a	0.07 \pm 0.01 a	0.07 \pm 0.01 a	0.07 \pm 0.01 a
Cd (mg/ kg dry soil)	0.13 \pm 0.01 a	0.13 \pm 0.01 a	0.11 \pm 0.01 b	0.11 \pm 0.01 b
Cu (mg/ kg dry soil)	3.64 \pm 0.48 b	3.36 \pm 0.71 b	5.43 \pm 0.27 a	5.17 \pm 0.31 a
Ni (mg/ kg dry soil)	1.24 \pm 0.11 a	1.25 \pm 0.07 a	0.46 \pm 0.05 b	0.44 \pm 0.04 b
Pb (mg/ kg dry soil)	5.32 \pm 0.33 b	5.01 \pm 0.19 b	7.66 \pm 1.02 a	5.75 \pm 0.27 b
Zn (mg/ kg dry soil)	3.71 \pm 0.72 a	2.69 \pm 0.41 b	2.62 \pm 0.24 b	1.76 \pm 0.16 c
Soil biology				
PHOS (mU/g dry soil)	24.43 \pm 2.62 a	23.65 \pm 3.85 a	15.69 \pm 1.08 b	20.29 \pm 1.80 a
GLU (mU/g dry soil)	10.01 \pm 1.09 c	8.91 \pm 1.35 bc	11.18 \pm 0.76 b	13.31 \pm 1.00 a
ARS (mU/g dry soil)	4.57 \pm 0.68 a	5.28 \pm 0.79 a	2.57 \pm 0.18 b	2.82 \pm 0.28 b
URE (mU/g dry soil)	5.63 \pm 0.71 a	5.20 \pm 0.88 a	4.11 \pm 0.43 b	6.33 \pm 0.17 a
ARN (mU/g dry soil)	2.09 \pm 0.74 b	1.69 \pm 0.92 b	11.13 \pm 0.42 a	11.80 \pm 1.18 a
Shannon.Enzy	1.28 \pm 0.05 b	1.27 \pm 0.10 b	1.44 \pm 0.02 a	1.45 \pm 0.02 a
SMB (μ g DNA/g dry soil)	28403 \pm 6660 ab	23672 \pm 1153 b	28688 \pm 3150 ab	34600 \pm 4062 a
Richness.Ba	1453 \pm 34 a	1371 \pm 35 a	1382 \pm 96 a	1314 \pm 210 a
Shannon.Ba	5.66 \pm 0.09 a	5.48 \pm 0.09 a	5.63 \pm 0.14 a	5.29 \pm 0.52 a
Evenness.Fu	0.78 \pm 0.01 a	0.76 \pm 0.01 a	0.78 \pm 0.03 a	0.74 \pm 0.06 a
Richness.Fu	912 \pm 137 a	973 \pm 189 a	961 \pm 173 a	1011 \pm 359 a
Shannon.Fu	4.42 \pm 0.17 a	4.51 \pm 0.21 a	4.02 \pm 0.53 a	4.46 \pm 0.49 a
Evenness.Fu	0.65 \pm 0.01 a	0.66 \pm 0.01 a	0.59 \pm 0.07 a	0.65 \pm 0.04 a
BFN (ind./100 g dry soil)	231 \pm 108 b	226 \pm 36 b	885 \pm 264 a	889 \pm 53 a
FFN (ind./100 g dry soil)	61 \pm 27 c	113 \pm 54 c	313 \pm 43 b	650 \pm 123 a
OPN (ind./100 g dry soil)	150 \pm 122 a	26 \pm 21 a	14.25 \pm 6 a	88 \pm 69 a
PPN (ind./100 g dry soil)	257 \pm 236 b	783 \pm 143 a	835 \pm 85 a	698 \pm 244 a
EI	49 \pm 19 c	60 \pm 6 bc	86 \pm 5 ab	76 \pm 5 a
SI	57 \pm 21 ab	33 \pm 10 a	29 \pm 11 c	28 \pm 16 c
NCR	79 \pm 5 a	69 \pm 9 ab	73 \pm 4 a	58 \pm 6 b
PPI	2.15 \pm 0.18 b	2.48 \pm 0.08 a	2.40 \pm 0.07 ab	2.59 \pm 0.22 a
MI	2.49 \pm 0.34 a	1.98 \pm 0.08 b	1.54 \pm 0.16 bc	1.78 \pm 0.22 c
Shannon.Nat	1.78 \pm 0.21 a	1.82 \pm 0.13 a	1.87 \pm 0.17 a	2.06 \pm 0.09 a

Table S11. Percentage of increase (%) in OW applications on soil enzyme, microbe, nematode and physical and chemical properties compared to the control in COL. Name of abbreviations can be found in the abbreviation table.

	SLU_N-	GWS_N-	BIO_N-	FYM_N-	FYMC_N-	SLU_N+	GWS_N+	BIO_N+	FYM_N+	FYMC_N+
TC	2.2	11.5	14.0	10.9	8.0	4.9	11.4	16.3	10.3	11.2
TN	2.3	13.4	18.2	7.9	6.6	6.3	15.0	17.6	12.1	11.9
CN	-0.2	-1.7	-3.5	2.8	1.5	-1.3	-3.2	-1.2	-1.7	-0.7
pH	-0.7	-0.9	-0.2	-0.1	0.1	-0.6	-1.1	-0.2	-0.6	-0.4
CEC	0.4	3.3	2.1	0.4	-0.4	2.1	3.5	3.1	1.3	1.5
EK	-18.0	-4.3	38.5	116.0	113.0	4.1	13.4	33.4	113.5	84.4
AP	104.4	142.5	28.7	57.5	61.9	118.1	107.2	11.6	52.2	38.4
Cr	-4.6	-7.5	12.8	1.7	-0.5	8.8	-9.9	9.3	-0.9	0.3
Cd	-1.6	0.5	3.6	-3.3	-1.6	1.4	8.8	6.5	3.5	5.1
Cu	7.4	5.0	-0.1	-4.4	-7.1	34.5	24.8	11.1	5.8	11.1
Ni	-3.9	3.2	14.2	8.0	4.4	-0.8	22.8	20.5	19.5	20.6
Pb	-18.0	-12.0	-5.4	-10.4	-17.3	1.7	5.1	16.0	8.9	8.0
Zn	29.0	64.2	55.5	34.5	27.6	66.9	100.9	70.1	54.0	44.9
Active.C	13.1	29.7	34.8	31.7	13.6	1.4	24.5	30.7	25.7	22.7
Stable.C	5.0	7.5	6.3	0.6	4.4	4.0	10.2	10.8	5.0	7.0
SC.ratio	-2.5	-6.5	-8.2	-9.3	-2.8	0.8	-4.5	-6.0	-6.7	-5.0
MWD.WS				41.0		-32.4	-17.8	-7.1	-22.4	-20.3
MWD.FW				-9.4		30.0	-5.0	41.9	-15.3	100.5
MWD.MB				-8.2		-7.6	-3.0	3.3	-11.1	-12.8
PHOS	15.0	8.2	16.3	11.5	15.1	-4.0	5.3	5.1	7.4	4.1
GLU	28.6	9.4	21.8	15.1	13.6	3.5	8.8	3.9	11.9	7.6

	SLU_N-	GWS_N-	BIO_N-	FYM_N-	FYMC_N-	SLU_N+	GWS_N+	BIO_N+	FYM_N+	FYMC_N+
ARS	25.4	12.0	23.7	24.8	16.6	0.5	6.9	-0.5	1.7	16.4
URE	12.4	4.0	22.6	30.5	24.4	2.6	3.4	5.4	17.2	10.3
ARN	13.9	-2.8	-0.9	-0.1	0.9	11.6	2.8	-0.4	8.3	3.6
Shannon.Enzy	0.2	0.0	-37.7	1.3	0.6	0.7	-0.1	-21.0	0.1	0.9
Simpson.Enzy	-0.1	-0.3	-34.0	0.4	0.0	0.8	-0.3	-16.0	0.1	0.4
SMB	51.6	56.5	32.0	51.2	40.6	10.9	2.8	5.8	35.7	14.0
Richness.Ba	-1.5	-7.9	-9.6	4.9	9.4	9.5	7.9	5.0	8.1	12.8
Shannon.Ba	-6.6	-1.5	-8.7	-1.7	1.4	4.8	4.1	4.2	6.7	8.9
Evenness.Fu	-6.4	-0.3	-7.4	-2.4	0.2	3.4	3.0	3.5	5.6	7.1
Richness.Fu	-15.3	-7.5	-21.0	-37.8	-26.2	-13.0	-21.3	-22.3	-16.0	-5.3
Shannon.Fu	4.6	3.0	1.4	-0.4	5.6	-2.2	-11.1	-7.9	-9.3	-9.9
Evenness.Fu	7.5	4.2	5.2	7.3	10.5	-0.5	-8.5	-5.0	-7.5	-9.5
BFN	-9.6	20.4	33.6	3.1	27.6	-36.6	-34.2	7.7	-43.5	5.2
FFN	-51.8	12.7	-18.6	-32.9	-22.8	-18.3	5.0	131.2	-20.9	148.2
OPN	-36.6	-13.6	-50.9	-39.5	-64.5	68.4	29.8	366.7	5.3	128.1
PFN	28.1	33.5	36.9	76.3	93.3	-11.3	-30.6	71.5	20.8	-1.3
EI	9.8	3.3	5.9	2.3	7.9	-6.7	-7.6	-7.0	-8.5	-8.8
SI	35.4	15.0	-2.7	0.0	-22.1	-22.8	-47.4	-5.3	10.5	-33.3
NCR	23.3	3.9	17.2	15.5	17.2	-7.2	-15.7	-15.4	-6.1	-19.8
PPI	-8.2	-11.2	-6.7	-12.3	-1.5	3.1	1.3	2.3	-3.1	2.0
MI	-7.9	-3.0	-7.2	-4.9	-10.8	6.3	4.9	9.1	13.0	7.5
Shannon.Nat	-3.6	-0.4	-7.8	-9.1	-9.1	4.8	9.1	5.9	2.8	-3.3

Table S12. Percentage of increase (%) in OW applications on soil enzyme, microbe, nematode and physical and chemical properties compared to the control in QUA. Name of abbreviations can be found in the abbreviation table.

	MSW_N	BIO_N	GWS_N	FYM_N	MSW_LEG	BIO_LEG	GWS_LEG	FYM_LEG
TC	38.7	63.7	70.7	42.9	40.7	66.3	73.0	46.4
TN	36.4	69.5	71.6	38.7	33.5	60.1	69.6	35.8
CN	1.7	-3.3	-0.5	3.2	5.3	3.9	1.8	7.6
pH	12.2	12.4	1.5	7.7	12.6	14.5	2.3	4.8
CEC	24.7	34.8	25.9	20.1	25.3	37.5	26.2	15.2
EK	50.4	129.7	84.0	261.5	16.1	73.0	39.7	184.1
AP	30.3	76.4	259.7	110.5	10.6	69.2	329.8	79.5
Cr	73.4	189.2	148.3	31.2	66.2	161.3	139.7	30.5
Cd	13.1	26.6	15.2	15.2	11.6	12.6	15.0	12.1
Cu	61.3	46.1	92.0	44.7	69.1	41.0	98.9	34.6
Ni	4.6	4.1	5.3	-2.0	6.3	6.0	8.6	-3.6
Pb	47.0	62.1	34.0	49.9	40.1	54.7	34.2	125.6
Zn	157.7	177.1	294.3	157.0	185.5	192.0	360.6	177.2
Active.C	83.4	120.4	154.3	101.8	80.3	120.4	178.2	113.1
Stable.C	10.5	27.1	43.0	12.8	14.7	30.2	25.1	13.3
SC.ratio	-19.4	-21.1	-22.3	-22.4	-16.7	-19.5	-29.9	-23.5
MWD.WS	22.2	41.5	63.7	46.9				-31.0
MWD.FW	30.6	23.5	9.1	9.1				-36.1
MWD.MB	8.6	2.8	4.1	-2.8				-5.1
PHOS	3.0	3.0	35.4	23.0	3.6	-4.6	46.7	10.8
GLU	48.2	45.2	42.7	52.4	47.1	37.7	61.7	44.3
ARS	3.7	-3.6	0.7	9.0	7.4	2.1	5.4	12.3
URE	57.2	47.2	38.8	47.6	51.8	53.9	53.9	63.8
ARN	164.8	125.9	149.1	125.8	166.8	167.9	145.1	116.8
Shannon.Enzy	10.3	8.2	3.0	5.2	10.4	11.9	2.8	8.6
Simpson.Enzy	9.4	7.7	1.7	4.5	7.4	8.7	-0.6	5.6
SMB	45.9	47.7	85.9	51.9	83.3	89.9	95.4	85.9
Richness.Ba	-1.1	4.5	8.8	6.6	2.5	5.0	1.6	6.3
Shannon.Ba	0.5	-0.7	0.0	0.5	0.3	1.7	-0.4	-1.5
Evenness.Fu	0.7	-1.3	-1.1	-0.3	-0.1	1.0	-0.6	-2.3
Richness.Fu	-5.3	18.2	15.2	-9.2	-7.8	-14.1	-8.9	3.9
Shannon.Fu	-1.3	3.3	6.3	-0.6	-3.0	-4.5	0.1	2.6
Evenness.Fu	-0.6	1.1	4.1	1.6	-2.0	-2.3	1.3	2.1
BFN	125.7	175.1	64.7	256.9	-40.3	8.5	32.1	-13.3
FFN	111.6	45.8	12.9	59.3	-18.0	58.2	31.1	-4.9
OPN	-27.2	-17.5	-31.1	151.5	-95.0	-95.3	-74.4	-92.1
PFN	26.9	26.9	0.8	44.0	0.9	45.8	104.7	9.5
EI	-10.0	0.0	11.3	23.8	40.0	45.1	38.5	39.0
SI	-56.2	-22.3	-19.2	-10.0	-64.8	-34.4	-26.4	-38.8
NCR	-1.5	13.1	8.0	18.9	-7.0	-9.2	-0.6	-1.9
PPI	-3.0	3.2	2.9	0.0	19.2	17.7	10.7	19.8
MI	-3.7	-4.0	-6.9	-11.0	-22.8	-24.3	-20.8	-22.7
Shannon.Nat	0.3	6.6	3.7	9.4	13.2	13.3	4.3	15.7

Table S13. Principal components, eigenvalues and component matrix variables. Name of abbreviations can be found in the abbreviation table.

Component	PC1	PC2	PC3	PC4	PC5	PC6	Communality	Weight
Eigenvalue	10.4	6.0	2.9	2.8	2.3	1.8		
Proportion of variance	29.8	17.1	8.2	8.1	6.6	5.1		
Cumulative variance %	29.8	46.9	55.1	63.2	69.7	74.8		
Factor loadings								
TC		0.87	0.20	0.29		0.19	0.93	0.00
TN		0.92	0.12	0.15	-0.12	0.13	0.91	0.18
CN	-0.46		0.29	0.56	0.19	0.10	0.65	0.00
pH	-0.86	0.16	0.19	0.30	0.11		0.91	0.00
CEC	-0.90	0.27	0.14	0.21			0.95	0.00
EK		0.18		0.83			0.73	0.17
AP	0.47	0.69	-0.14				0.73	0.00
Zn	0.77	0.56					0.93	0.00
Pb	0.44	0.10	0.11	0.27		0.48	0.52	0.00
Ni	0.94			-0.24			0.94	0.19
Cu	-0.21	0.75	0.14	-0.18	0.18		0.70	0.00
Cd	0.87	0.22					0.82	0.00
Cr	0.68	0.48		-0.23		0.19	0.78	0.00
Active.C	0.64	0.72				0.11	0.94	0.00
Stable.C	-0.36	0.76	0.15			0.13	0.76	0.00
SC.ratio	-0.78	-0.49		-0.11			0.88	0.00
PHOS	0.66	0.29			-0.25	-0.28	0.66	0.00
GLU		0.61	0.21	0.27		-0.28	0.57	0.00
ARS	0.88	-0.10	-0.11	-0.17			0.83	0.00
URE	0.67	0.32	0.25		0.19		0.65	0.00
ARN	-0.88	0.24	0.16	0.20	0.16		0.92	0.00
Shannon.Enzy					0.95		0.91	0.18
Simpson.Enzy	-0.14				0.95		0.93	0.00
SMB	0.25	0.64	0.12	0.23	0.23	0.12	0.61	0.00
Richness.Fu	0.20			-0.39		0.10	0.20	0.00
BFN	-0.58	0.13		0.55	-0.16	0.11	0.70	0.00
FFN	-0.64		-0.12	0.26	-0.17	0.42	0.71	0.00
OPN	-0.14	-0.17	-0.87			0.13	0.84	0.17
PFN	-0.29	0.19	-0.13	0.64	-0.22		0.60	0.00
EI	-0.56	0.21	0.43	0.28	0.15		0.64	0.00
SI	0.16	-0.14	-0.78				0.65	0.00
NCR	0.58			0.10		-0.47	0.58	0.00
PPI	0.18		0.59			0.55	0.69	0.00
MI	0.48	-0.26	-0.72	-0.28			0.89	0.00
Shannon.Nat		0.13			0.20	0.66	0.52	0.10

Appendix III

An 18-year field experiment to assess how various types of organic waste used at European regulatory rates sustain crop yields and C, N, P, and K dynamics in a French calcareous soil

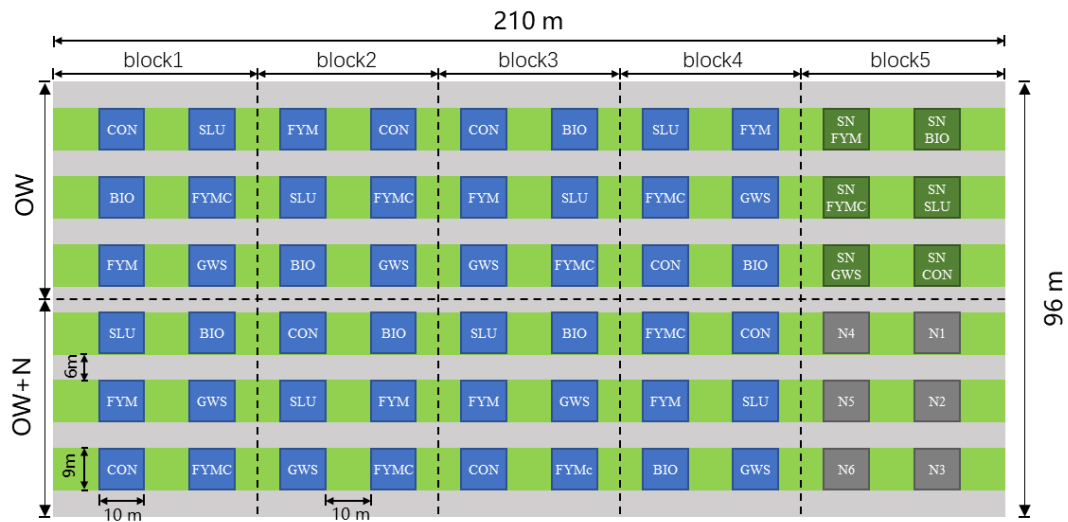


Figure. S1 Long-term field experiment PROspective located in Colmar (SN: bare fallow treatments, N1 to N6: plots with either mineral N only or no fertilization for the computation of apparent nitrogen recovery; SLU: dehydrated sludge; GWS: green waste and sewage sludge compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control), OW+N: optimal N fertilization, OW: no mineral fertilization until 2014 and digestate application after 2015.

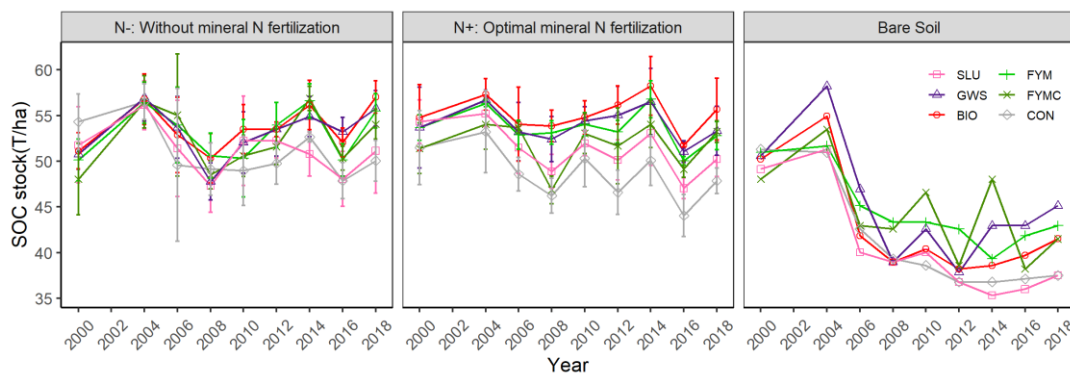


Figure. S2 SOC stocks under the different treatments of OW application in Colmar. SOC stocks were computed with the measured C contents instead of with N contents and the C/N ratio in the main manuscript. The influence of carbonates on the C content analysis introduced noise to the trends of SOC evolution (SLU: dehydrated sludge; GWS: green waste and sewage sludge compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control). Error bars are the standard deviations (four replicates) (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

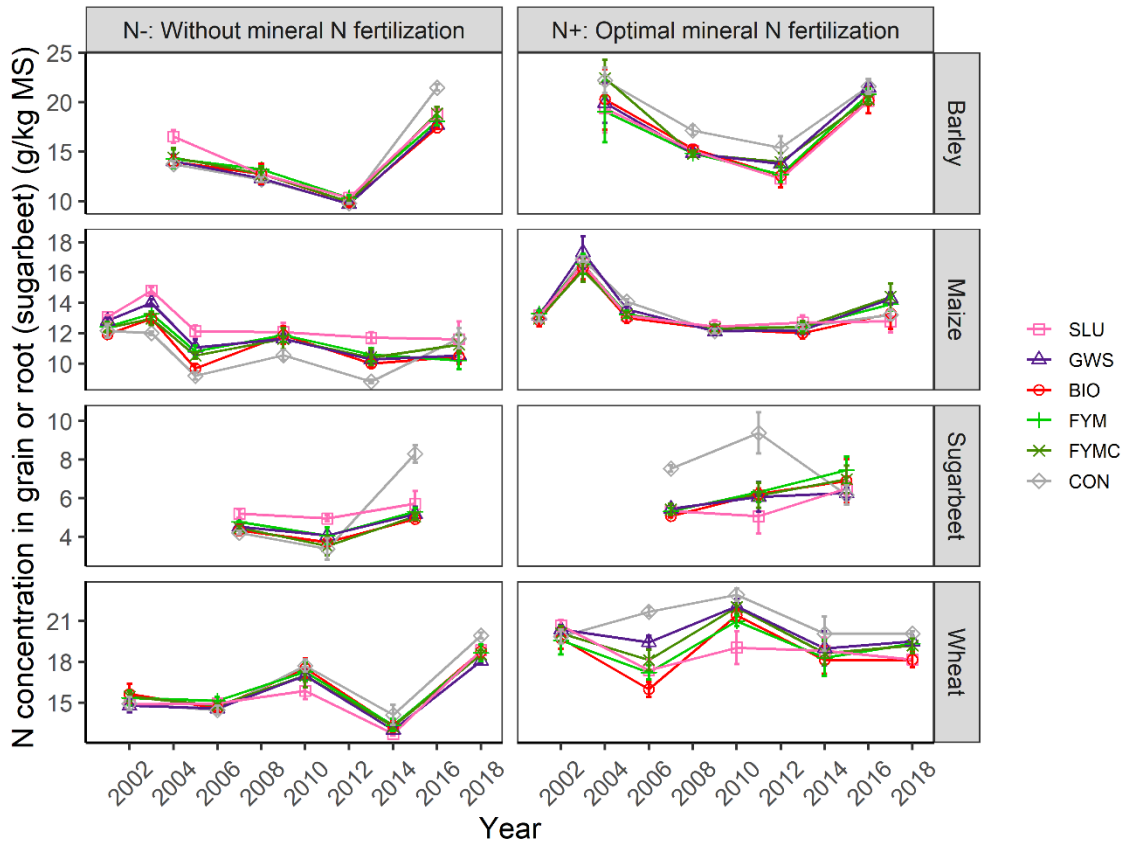


Figure. S3 Concentrations of N in barley grain, maize grain, sugarbeet root and wheat grain from 2001 to 2018 under the 12 treatments (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: dairy farmyard manure; FYMC: FYM after composting; CON: control; and N+: optimal dose of mineral N fertilization and N-: no mineral N fertilization until 2014 and digestate from 2015). Error bars are the standard deviations (four replicates).

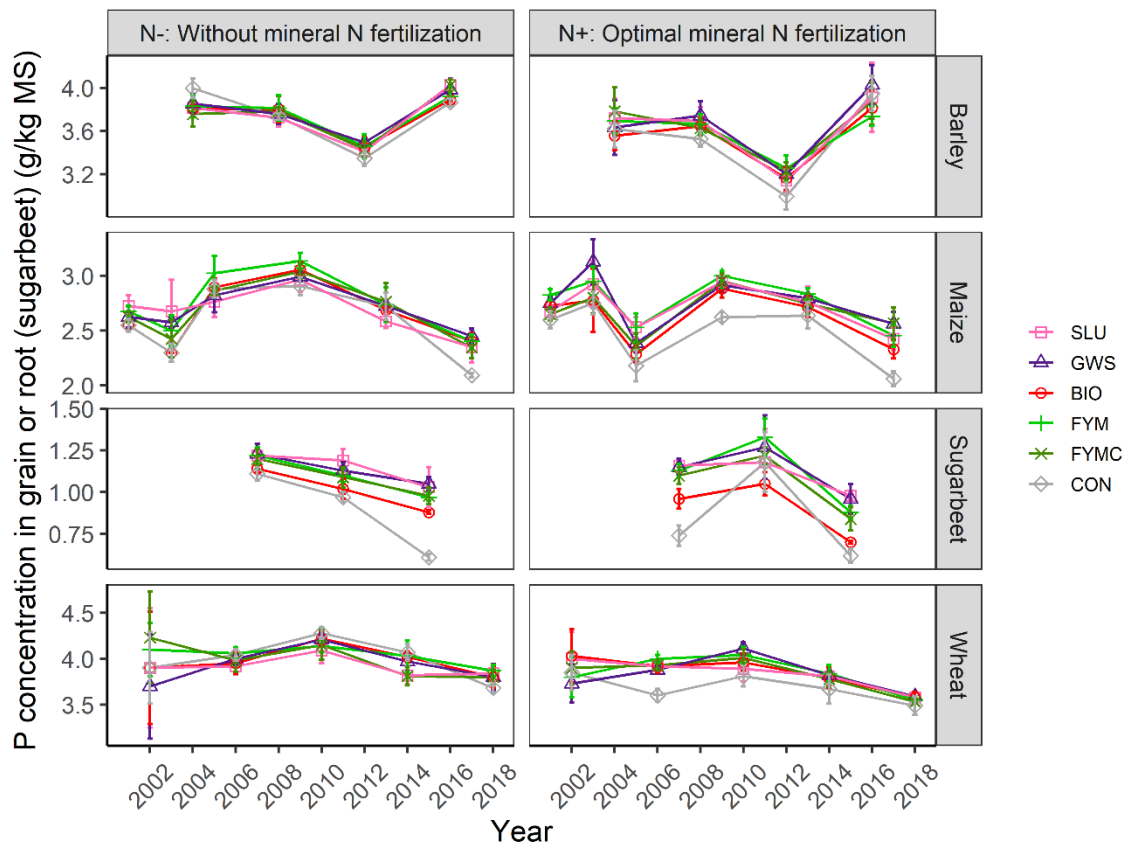


Figure. S4 Concentrations of P in barley grain, maize grain, sugarbeet root and wheat grain from 2001 to 2018 under the 12 treatments (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: dairy farmyard manure; FYMC: FYM after composting; CON: control; N+: optimal dose of mineral N fertilization; N-: no mineral N fertilization). Error bars are the standard deviations (four replicates)

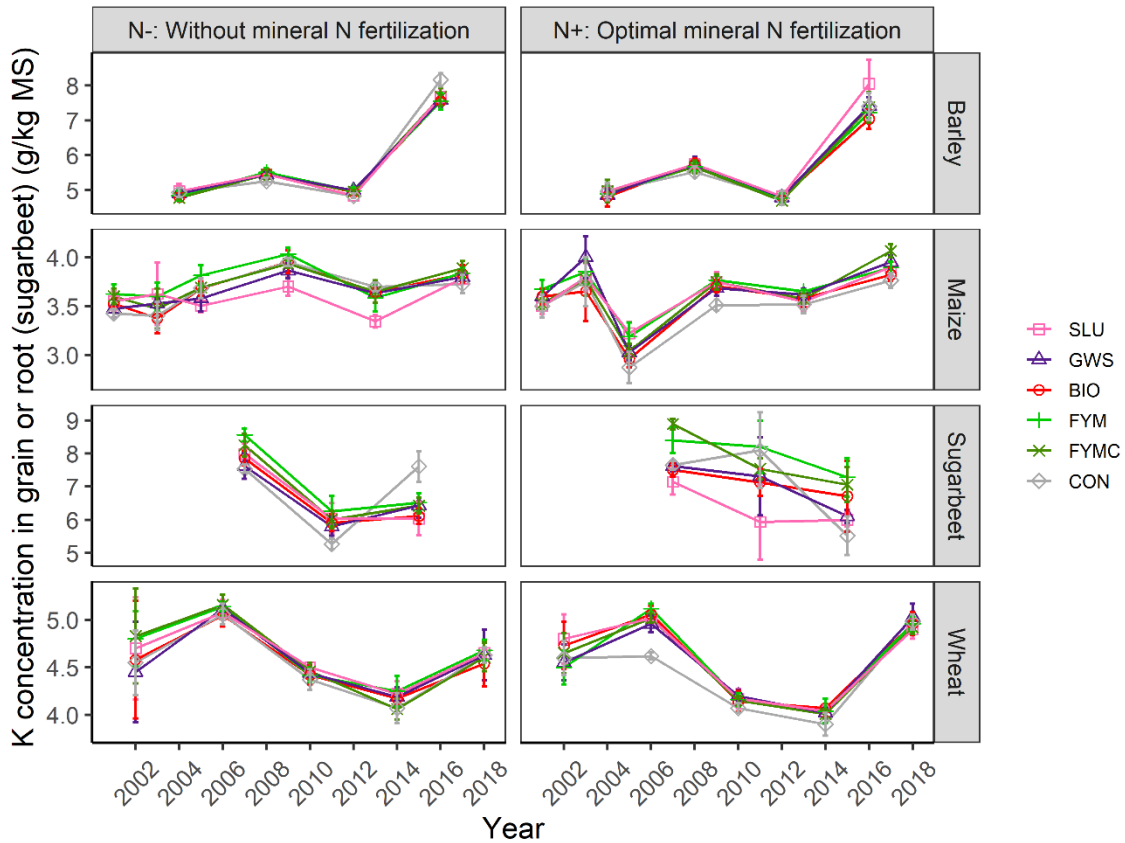


Figure. S5 Concentrations of K in barley grain, maize grain, sugarbeet root and wheat grain from 2001 to 2018 under the 12 treatments (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: dairy farmyard manure; FYMC: FYM after composting; CON: control; N+: optimal dose of mineral N fertilization; and N-: no mineral N fertilization). Error bars are the standard deviations (four replicates)

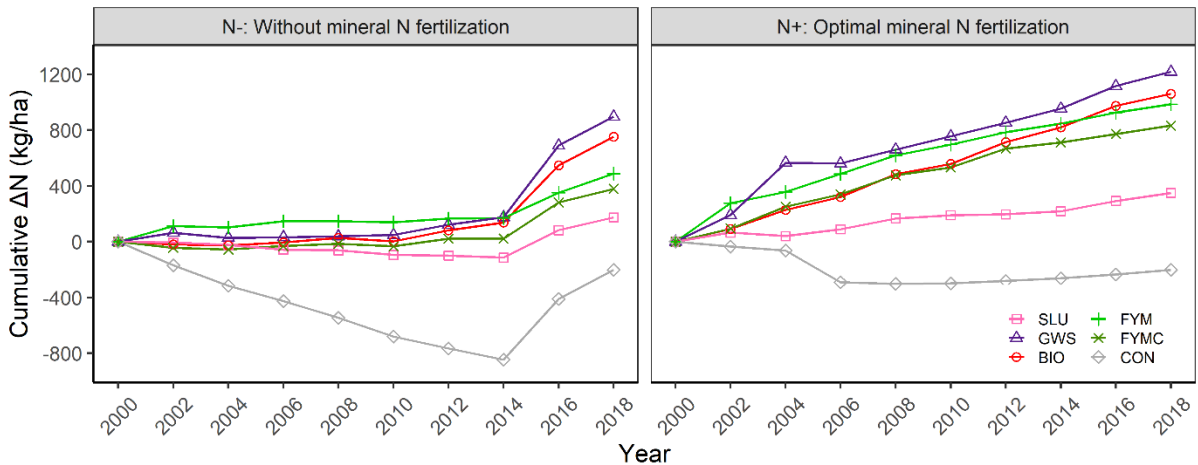


Figure. S6. Cumulative ΔN in the soil plowed layer for the different CON and OW treatments in the N- and N+ experiments during the 18 years of experimentation (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control). Error bars are the standard deviations (four replicates) (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

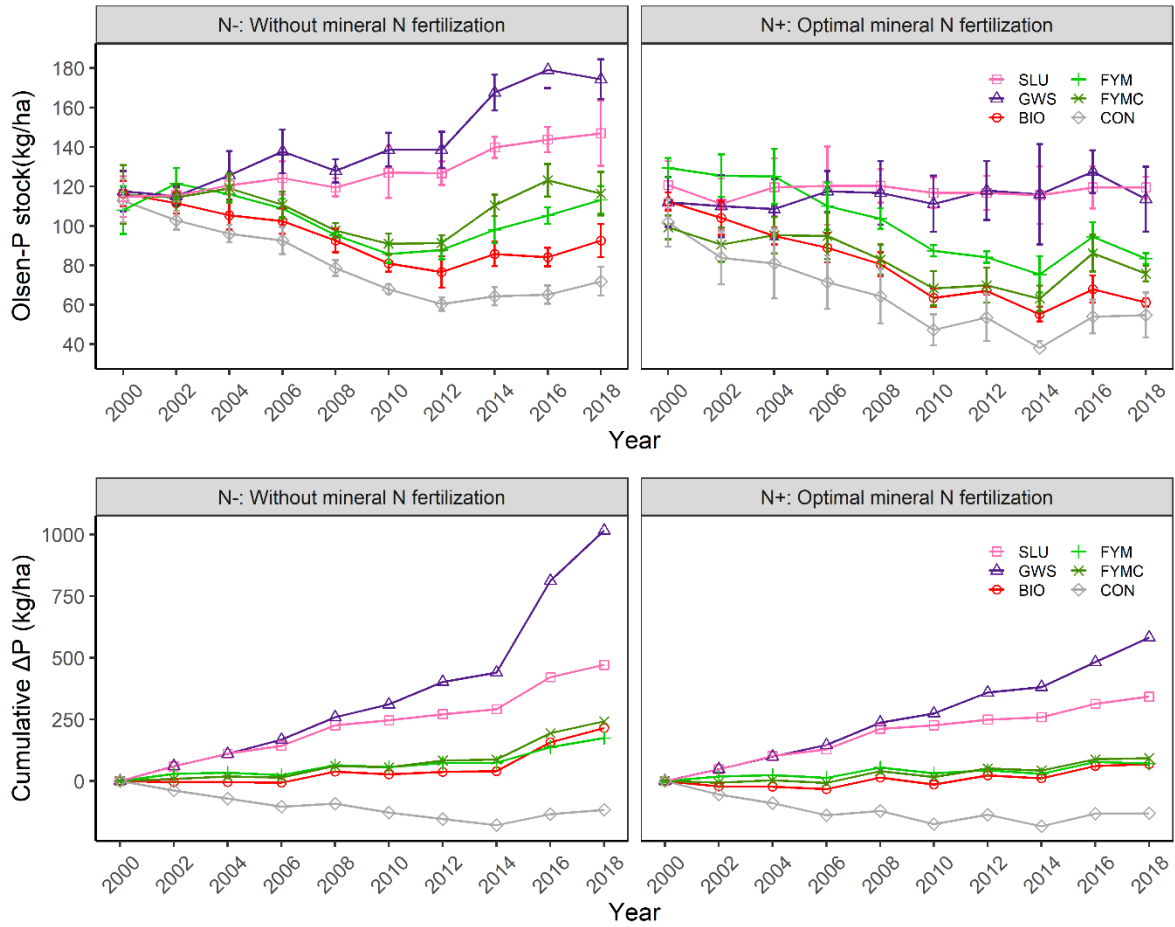


Figure. S7. Cumulative ΔP and Olsen-P concentration in the soil plowed layer for the different CON and OW treatments in the N- and N+ experiments during the 18 years of experimentation (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control) Error bars are the standard deviations (four replicates) (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

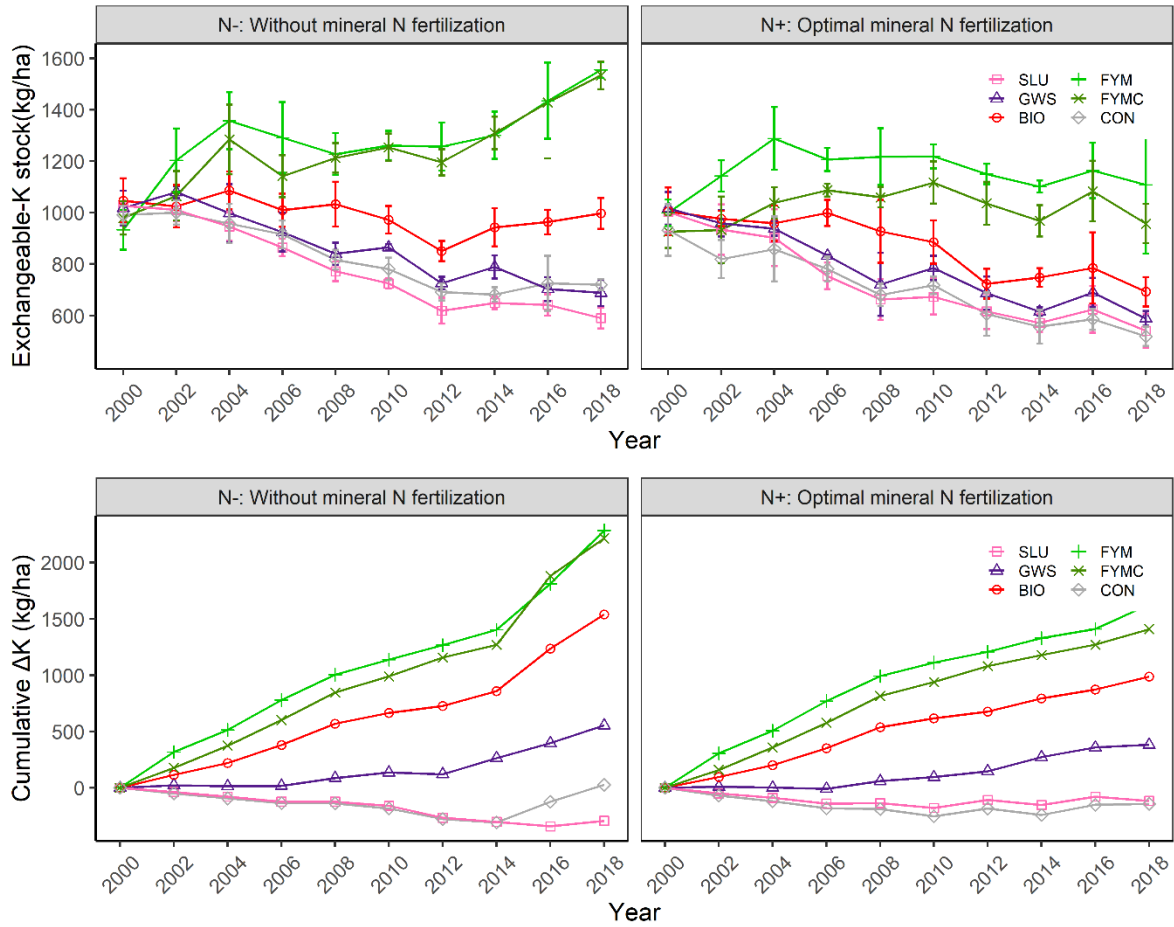


Figure. S8. Cumulative ΔK and exchangeable K concentration in the soil plowed layer for the different CON and OW treatments in the N- and N+ experiments during the 18 years of experimentation (SLU: dehydrated urban sewage sludge; GWS: green waste and SLU compost; BIO: biowaste compost; FYM: farmyard manure; FYMC: farmyard manure compost; and CON: control). Error bars are the standard deviations (four replicates) (for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Table S1: The management log of the experiment.

Date	Crop	Cultivation	Soil analysis	OW application	Mineral N application
2000	-	-	09/19	-	-
2001	Maize	2001.05 - 2001.10	-	05/14	06/19
2002	Wheat	2001.11 - 2002.07	11/06	-	03/11, 04/11
2003	Maize	2003.04 - 2003.09	-	02/06	06/18
2004	Barley	2004.03 - 2004.07	12/09	-	-
2005	Maize	2005.04 - 2005.10	-	01/25	06/17
2006	Wheat	2005.10 - 2006.07	12/12	-	03/23, 04/04
2007	Sugar beet	2007.03 - 2007.09	-	02/05	03/29, 05/29
2008	Barley	2008.03 - 2008.07	09/29	-	04/24
2009	Maize	2009.04 - 2009.09	-	02/17	06/02
2010	Wheat	2009.11 - 2010.07	11/24	12/13	04/12, 05/19
2011	Sugar beet	2011.04 - 2011.10	-	-	04/01, 04/27
2012	Barley	2012.03 - 2012.07	07/24	-	04/25
2013	Maize	2013.04 - 2013.10	-	02/20	06/24
2014	Wheat	2013.11 - 2014.07	07/24	07/31	04/16, 05/16
	Mustard (CC)	2014.08 - 2014.10			
2015	Sugar beet	2015.03 - 2015.10	-	-	03/05
2016	Barley	2016.03 - 2016.07	07/21	07/27	04/22
	Mustard (CC)	2016.07 - 2016.11			
2017	Maize	2017.03 - 2017.09	-	-	05/31
2018	Wheat	2018.03 - 2018.07	07/20	07/31	03/26, 04/09

Table S2: Amount of applied organic waste (OW) over the 2001-2013 period in the N+ and N- treatments (mean \pm sd).

OW	Applica tion period (year. month)	Quantity (t ha ⁻¹ FM)	Organic C (kg ha ⁻¹ DM)	Total N (kg ha ⁻¹ DM)	Mineral N (kg ha ⁻¹ DM)	P₂O₅ (kg ha ⁻¹ DM)	K₂O (kg ha ⁻¹ DM)
SLU	2001.5	18.7	1098	201	36	242	26
SLU	2003.2	16.4	1077 \pm 20	185 \pm 2	19 \pm 6	211 \pm 8	15 \pm 0
SLU	2005.1	14.2	1003 \pm 9	153 \pm 1	57 \pm 4	177 \pm 5	16 \pm 0
SLU	2007.2	16.3	922 \pm 12	147 \pm 3	51 \pm 4	176 \pm 5	17 \pm 0
SLU	2009.2	13.8	1021 \pm 120	16 \pm 20	53 \pm 1	156 \pm 19	26 \pm 3
SLU	2010.12	12.6	826 \pm 16	128 \pm 1	38 \pm 1	143 \pm 1	18 \pm 2
SLU	2013.2	12.9	847 \pm 14	150 \pm 3	37 \pm 2	138 \pm 3	16 \pm 1
GWS	2001.5	21.0	3744	263	27	237	95
GWS	2003.2	12.5	2443 \pm 21	141 \pm 1	25 \pm 2	201 \pm 3	52 \pm 1
GWS	2005.1	16.8	2084 \pm 82	164 \pm 12	38 \pm 4	230 \pm 19	68 \pm 6
GWS	2007.2	22.9	1665 \pm 58	144 \pm 2	21 \pm 2	194 \pm 8	104 \pm 3
GWS	2009.2	15.3	1524 \pm 109	172 \pm 7	27 \pm 1	213 \pm 6	121 \pm 4
GWS	2010.12	11.6	1662 \pm 62	183 \pm 2	44 \pm 1	288 \pm 5	123 \pm 2
GWS	2013.2	11.2	1995 \pm 47	180 \pm 4	20 \pm 3	167 \pm 7	225 \pm 4
BIO	2001.5	11.1	2407	158	3	81	201 \pm 3
BIO	2003.2	10.7	2165 \pm 218	166 \pm 12	2 \pm 1	83 \pm 3	187 \pm 5
BIO	2005.1	16.6	2151 \pm 55	159 \pm 4	4 \pm 1	87 \pm 14	256 \pm 6
BIO	2007.2	26.8	1915 \pm 60	164 \pm 5	7 \pm 1	82 \pm 5	240 \pm 9
BIO	2009.2	14.6	1386 \pm 29	128 \pm 4	8 \pm 1	62 \pm 3	173 \pm 5
BIO	2010.12	21.1	1949 \pm 26	181 \pm 5	12 \pm 0	96 \pm 6	210 \pm 4
BIO	2013.2	18.8	2101 \pm 30	173 \pm 5	8 \pm 1	82 \pm 3	208 \pm 4
FYM	2001.5	19.4	3188	301	32	166	453
FYM	2003.2	19.1	2900 \pm 215	173 \pm 17	5 \pm 1	99 \pm 13	301 \pm 37
FYM	2005.1	14.9	3170 \pm 75	215 \pm 14	14 \pm 3	89 \pm 5	391 \pm 11
FYM	2007.2	10.0	2552 \pm 150	156 \pm 19	7 \pm 1	83 \pm 12	314 \pm 48
FYM	2009.2	9.3	2877 \pm 121	163 \pm 1	6 \pm 1	81 \pm 1	226 \pm 14
FYM	2010.12	9.6	2092 \pm 48	147 \pm 6	22 \pm 12	121 \pm 6	306 \pm 21
FYM	2013.2	32.5	2219 \pm 34	140 \pm 6	12 \pm 5	87 \pm 9	221 \pm 16
FYMC	2001.5	22.3	2020	142	3	118	281
FYMC	2003.2	25.2	2408 \pm 65	164 \pm 3	2 \pm 0	108 \pm 5	299 \pm 4
FYMC	2005.1	41.6	2780 \pm 29	188 \pm 0	6 \pm 2	91 \pm 2	343 \pm 5
FYMC	2007.2	34.2	1987 \pm 49	154 \pm 4	2 \pm 0	94 \pm 3	323 \pm 8
FYMC	2009.2	41.8	1888 \pm 16	141 \pm 4	2 \pm 1	79 \pm 2	231 \pm 5
FYMC	2010.12	34.4	1929 \pm 96	158 \pm 8	2 \pm 0	141 \pm 6	344 \pm 17
FYMC	2013.2	36.9	1980 \pm 35	133 \pm 2	3 \pm 1	94 \pm 2	190 \pm 13

Table S3: Amount of applied organic waste (OW) over the 2014-2018 period.

Treatments	Applica tion period (year.m onth)	Quantity (t ha ⁻¹ FM)	Organic C (kg ha ⁻¹ DM)	Total N (kg ha ⁻¹ DM)	Mineral N (kg ha ⁻¹ DM)	P ₂ O ₅ (kg ha ⁻¹ DM)	K ₂ O (kg ha ⁻¹ DM)
SLU_N-	2014.7	21.0	1576 ± 276	231 ± 46	142 ± 8	334 ± 6	40 ± 1
SLU_N-	2016.7	12.5	878 ± 7	137 ± 2	64 ± 3	146 ± 4	19 ± 0
GWS_N-	2014.7	16.8	5601 ± 435	676 ± 88	116 ± 17	931 ± 51	336 ± 2
GWS_N-	2016.7	22.9	2372 ± 123	242 ± 10	82 ± 6	504 ± 22	153 ± 5
BIO_N-	2014.7	15.3	6715 ± 246	564 ± 127	52 ± 1	336 ± 5	625 ± 4
BIO_N-	2016.7	11.6	3229 ± 112	244 ± 3	53 ± 1	168 ± 3	329 ± 12
FYM_N-	2014.7	11.2	6613 ± 1103	350 ± 38	114 ± 3	221 ± 50	681 ± 83
FYM_N-	2016.7	11.1	4695 ± 331	179 ± 17	31 ± 5	122 ± 17	532 ± 28
FYMC_N-	2014.7	10.7	4196 ± 119	286 ± 82	11 ± 0	280 ± 43	844 ± 43
FYMC_N-	2016.7	16.6	1891 ± 52	157 ± 11	2 ± 0	150 ± 12	369 ± 19
SLU_N+	2014.7	11.9	891 ± 156	131 ± 26	80 ± 8	189 ± 3	22 ± 1
SLU_N+	2016.7	13.0	915 ± 7	143 ± 2	67 ± 3	153 ± 4	20 ± 0
GWS_N+	2014.7	11.9	1750 ± 136	211 ± 27	36 ± 17	291 ± 16	105 ± 1
GWS_N+	2016.7	22.9	1484 ± 77	152 ± 6	52 ± 6	316 ± 14	96 ± 3
BIO_N+	2014.7	15.3	2243 ± 82	188 ± 42	17 ± 1	112 ± 2	209 ± 1
BIO_N+	2016.7	11.6	2021 ± 72	153 ± 2	33 ± 1	105 ± 2	206 ± 7
FYM_N+	2014.7	11.2	2492 ± 416	132 ± 14	43 ± 3	83 ± 19	257 ± 31
FYM_N+	2016.7	11.1	2938 ± 207	112 ± 10	19 ± 5	76 ± 11	333 ± 17
FYMC_N+	2014.7	10.7	1431 ± 41	97 ± 28	4 ± 0	95 ± 15	288 ± 15
FYMC_N+	2016.7	16.6	1184 ± 33	98 ± 7	2 ± 0	94 ± 7	231 ± 12

Table S4: Amount of applied digestate over the 2014-2018 period.

Treatments	Applica tion period (year.m onth)	Quantity (t ha ⁻¹ FM)	Organic C (kg ha ⁻¹ DM)	Total N (kg ha ⁻¹ DM)	Mineral N (kg ha ⁻¹ DM)	P ₂ O ₅ (kg ha ⁻¹ DM)	K ₂ O (kg ha ⁻¹ DM)
SLU_N-	2016.3	16.0	408 ± 9	140 ± 4	60 ± 9	41 ± 1	77 ± 1
SLU_N-	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
GWS_N-	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
BIO_N-	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
FYM_N-	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
FYMC_N-	2016.3	16.0	408 ± 9	140 ± 4	60 ± 9	41 ± 1	77 ± 1
FYMC_N-	2018.4	25.6	620 ± 16	153 ± 4	123 ± 3	61 ± 3	108 ± 2
CON_N-	2015.3	60.0	1348 ± 11	436 ± 22	226 ± 33	88 ± 5	309 ± 11
CON_N-	2016.3	26.7	681 ± 15	234 ± 6	99 ± 15	69 ± 2	129 ± 2
CON_N-	2017.5	26.0	783 ± 15	229 ± 28	91 ± 13	67 ± 1	132 ± 6
CON_N-	2018.4	29.5	715 ± 18	176 ± 5	142 ± 3	70 ± 3	124 ± 3

Table S5. Mean chemical fertilizer application from 2001 to 2018 under the 12 treatments.

Treatments	N (kg yr ⁻¹ ha ⁻¹)	P ₂ O ₅ (kg yr ⁻¹ ha ⁻¹)	K ₂ O (kg yr ⁻¹ ha ⁻¹)
SLU_N-	0	5.8	9.7
GWS_N-	0	5.8	9.7
BIO_N-	0	5.8	9.7
FYM_N-	0	5.8	9.7
FYMC_N-	0	5.8	9.7
CON_N-	0	5.8	9.7
SLU_N+	48.7	5.8	34.5
GWS_N+	88.4	5.8	24.0
BIO_N+	83.6	12.8	13.5
FYM_N+	78.6	10.5	9.7
FYMC_N+	87.2	11.2	9.7
CON_N+	106.0	28.9	40.2

Table S6. Parameters used to calculate aboveground and belowground C inputs and crop residue humification coefficients (Clivot et al., 2019).

Crop type	Relative plant C allocation coefficients				Humification coefficients	
	R _P	R _S	R _R	R _E	Aerial	Root
Maize	0.426	0.363	0.128	0.083	0.233	0.4
Wheat	0.402	0.418	0.109	0.071	0.217	0.4
Barley	0.440	0.423	0.083	0.054	0.222	0.4
Sugarbeet	0.766	0.219	0.016	0.000	0.315	0.4
Mustard	0.000	0.791	0.127	0.082	0.311	0.4

Table S7. Mean harvested index (%) from 2001 to 2018 under the 12 treatments. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$.

Treatments	Maize	Wheat	Barley	Sugarbeet
SLU_N-	0.52±0.04 a	0.48±0.03 a	0.48±0.08 a	0.73±0.11 a
GWS_N-	0.52±0.04 a	0.47±0.04 a	0.49±0.06 a	0.74±0.10 a
BIO_N-	0.50±0.05 a	0.50±0.02 a	0.50±0.06 a	0.75±0.10 a
FYM_N-	0.51±0.04 a	0.48±0.02 a	0.49±0.06 a	0.73±0.11 a
FYMC_N-	0.51±0.04 a	0.48±0.02 a	0.48±0.08 a	0.73±0.11 a
CON_N-	0.51±0.03 a	0.48±0.03 a	0.47±0.08 a	0.73±0.11 a
SLU_N+	0.52±0.04 a	0.48±0.03 a	0.48±0.09 a	0.73±0.09 a
GWS_N+	0.51±0.04 a	0.47±0.02 a	0.46±0.10 a	0.72±0.10 a
BIO_N+	0.51±0.03 a	0.49±0.03 a	0.47±0.09 a	0.70±0.07 a
FYM_N+	0.50±0.04 a	0.47±0.05 a	0.46±0.10 a	0.70±0.07 a
FYMC_N+	0.51±0.03 a	0.47±0.03 a	0.47±0.09 a	0.69±0.10 a
CON_N+	0.52±0.03 a	0.48±0.02 a	0.46±0.11 a	0.67±0.10 a
OW_N- vs OW_N+	ns	ns	ns	***

Table S8. Mean harvested biomass from 2015 to 2018 under the 12 treatments. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$.

Treatments	Maize (t ha ⁻¹)	Wheat (t ha ⁻¹)	Sugar beet (t ha ⁻¹)	Barley (t ha ⁻¹)
SLU_N-	7.8±1.1 abc	5.8±0.2 cde	17.8±2.6 abcd	3.9±0.6 a
GWS_N-	8.5±0.2 a	5.5±0.4 e	18.4±1.1 abc	3.7±0.1 ab
BIO_N-	8.2±0.7 ab	5.7±0.4 de	18.7±0.3 abc	3.5±0.3 abc
FYM_N-	8.3±0.3 a	5.9±0.4 bcde	19.9±0.4 ab	3.6±0.4 abc
FYMC_N-	8.5±0.5 a	6.3±0.4 abc	19.6±0.4 ab	3.6±0.3 abc
CON_N-	8.2±0.7 ab	6.6±0.1 a	20.4±2.1 a	3.1±0.7 bcd
SLU_N+	6.7±0.7 cd	5.9±0.2 bcde	17.1±1.7 bcdef	3.0±0.3 bcd
GWS_N+	6.2±0.8 d	6.0±0.2 bcde	15.6±1.5 def	2.8±0.3 cd
BIO_N+	6.9±0.8 bcd	6.1±0.3 bcd	14.4±0.7 f	3.3±0.3 abcd
FYM_N+	6.8±0.3 cd	6.3±0.2 ab	15.0±1.7 ed	3.3±0.5 abcd
FYMC_N+	5.9±1.2 d	6.1±0.4 bcd	17.6±1.4 abcde	3.1±0.4 bcd
CON_N+	7.1±0.6 abcd	6.0±0.1 bcde	16.6±0.9 cdef	2.6±0.6 d
OW_N- vs OW_N+	***	ns	***	***

Abbreviations: NS, not significant; ***, $P < 0.001$.

Table S9. Mean harvested crop nutrient concentrations from 2001 to 2018 under the 12 treatments. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$.

(g kg ⁻¹)	Maize			Wheat			Barley			Sugarbeet		
	N	P	K	N	P	K	N	P	K	N	P	K
SLU_N-	12.6±1.1 abcd	2.7±0.2 a	3.6±0.1 a	15.5±2.0 b	3.9±0.1 ab	4.6±0.3 a	14.6±3.3 a	3.7±0.2 a	5.7±1.1 a	5.3±0.3 a	1.1±0.1 a	6.7±0.9 a
GWS_N-	11.7±1.3 bcd	2.7±0.2 a	3.6±0.1 a	15.5±1.8 b	3.9±0.2 ab	4.6±0.3 a	13.4±2.9 a	3.8±0.2 a	5.7±1.1 a	4.6±0.5 a	1.1±0.1 a	6.6±0.8 a
BIO_N-	11.1±1.2 d	2.6±0.3 a	3.7±0.2 a	16.0±2.0 b	4.0±0.1 a	4.6±0.3 a	13.5±2.7 a	3.7±0.2 a	5.7±1.1 a	4.3±0.5 a	1.0±0.1 a	6.6±0.9 a
FYM_N-	11.5±1.1 cd	2.7±0.3 a	3.7±0.2 a	16.0±1.8 b	4.0±0.1 a	4.7±0.3 a	14.0±2.8 a	3.8±0.2 a	5.7±1.1 a	4.7±0.5 a	1.1±0.1 a	7.1±1.0 a
FYMC_N-	11.5±0.9 cd	2.7±0.2 a	3.7±0.2 a	15.8±1.8 b	4.0±0.2 a	4.6±0.4 a	14.0±3.2 a	3.7±0.2 a	5.7±1.2 a	4.3±0.6 a	1.1±0.1 a	6.9±1.0 a
CON_N-	10.7±1.3 d	2.6±0.3 a	3.6±0.2 a	16.2±2.3 b	4.0±0.2 a	4.5±0.3 a	14.3±4.4 a	3.7±0.2 a	5.8±1.4 a	5.3±2.2 a	0.9±0.2 a	6.8±1.1 a
SLU_N+	13.5±1.4 ab	2.7±0.2 a	3.6±0.2 a	18.8±1.1 a	3.8±0.1 ab	4.6±0.4 a	16.7±3.2 a	3.6±0.3 a	5.9±1.3 a	5.6±0.6 ab	1.1±0.1 a	6.4±0.6 a
GWS_N+	13.8±1.8 a	2.8±0.2 a	3.6±0.3 a	20.1±1.1 a	3.8±0.2 ab	4.6±0.4 a	17.5±3.3 a	3.7±0.3 a	5.7±1.1 a	5.9±0.3 ab	1.1±0.1 a	7.0±0.6 a
BIO_N+	13.3±1.4 abc	2.6±0.2 a	3.6±0.3 a	18.7±1.8 a	3.9±0.2 ab	4.6±0.4 a	17.1±3.3 a	3.5±0.2 a	5.6±0.9 a	6.1±0.8 ab	0.9±0.1 a	7.1±0.3 a
FYM_N+	13.6±1.4 ab	2.8±0.2 a	3.7±0.2 a	19.1±1.3 a	3.8±0.2 ab	4.6±0.4 a	16.9±3.2 a	3.6±0.2 a	5.6±1.0 a	6.4±0.9 ab	1.1±0.2 a	8.0±0.5 a
FYMC_N+	13.6±1.3 ab	2.7±0.2 a	3.6±0.3 a	19.6±1.4 a	3.8±0.2 ab	4.6±0.4 a	17.9±3.6 a	3.6±0.2 a	5.7±1.0 a	6.2±0.6 ab	1.1±0.2 a	7.8±0.8 a
CON_N+	13.6±1.6 ab	2.5±0.3 a	3.5±0.3 a	20.9±1.2 a	3.7±0.1 b	4.4±0.4 a	19.1±2.9 a	3.5±0.3 a	5.7±1.0 a	7.7±1.3 a	0.8±0.2 a	7.1±1.1 a
OW_N+ vs OW_N-	***	ns	ns	***	***	ns	***	*	ns	***	ns	ns

Abbreviations: NS, not significant; ***, $P < 0.001$.

Table S10. Mean C stocks and C stocks change in the different treatments. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$.

Treatments	C stocks 2001 (t ha ⁻¹)	C stocks 2018 (t ha ⁻¹)	C stocks Change (t ha ⁻¹)
SLU_N-	52.4±2.1 abc	50.9±3.1 de	-1.4±1.5 e
GWS_N-	51.0±2.3 bc	56.4±0.8 ab	5.4±1.0 a
BIO_N-	51.8±1.9 abc	58.8±1.5 a	7.0±1.4 a
FYM_N-	50.5±2.8 bc	53.7±1.3 bcd	3.2±0.7 b
FYMC_N-	50.1±1.8 c	53.0±2.4 cd	2.9±0.8 bc
CON_N-	53.3±1.8 abc	49.8±1.7 ef	-3.6±1.8 f
SLU_N+	55.1±1.6 a	50.6±1.5 de	-4.4±1.1 f
GWS_N+	53.6±1.4 abc	54.8±1.8 bc	1.2±1.7 bcd
BIO_N+	54.8±0.8 a	56.0±1.4 abc	1.3±0.8 bcd
FYM_N+	54.1±1.6 ab	53.4±1.4 bcd	-0.7±1.7 de
FYMC_N+	52.6±1.4 abc	53.3±0.7 bcd	0.8±1.1 cd
CON_N+	52.2±2.1 abc	47.6±1.0 f	-4.5±0.6 f

Table S11. Mean SMN stocks (0-120 cm) during the 2001-2013 and 2014-2018 periods in the different treatments. Values with the same lowercase letters in a row are not significantly different at $P < 0.05$.

Treatments	2001-2013		2014-2018	
	1st year	2nd year	1st year	2nd year
SLU_N-	49.8±13.8a	48.1±14.8a	42.8±1.2a	66.9±17.3a
GWS_N-	35.5±10.2b	44.8±13.1a	40.8±2.2a	52.9±5.5a
BIO_N-	31.9±7.0b	47.0±14.7a	39.4±2.3a	49.9±11.3a
FYM_N-	37.6±10.3b	52.6±18.0a	36.5±1.4a	66.2±7.3a
FYMC_N-	35.7±8.0b	46.7±14.3a	41.2±8.5a	67.0±11.2a
CON_N-	28.3±6.8b	44.9±13.8a	33.1±7.8a	102.5±34.7a
SLU_N+	47.6±16.5a	46.6±12.2a	40.4±12.3a	77.1±11.4a
GWS_N+	47.0±16.3a	47.0±13.1a	44.6±8.0a	104.8±47.6a
BIO_N+	47.3±23.5a	50.8±12.4a	37.6±5.4a	101.0±38.4a
FYM_N+	44.8±15.4a	49.3±10.9a	43.0±2.7a	110.5±41.6a
FYMC_N+	44.4±12.8a	54.4±16.6a	45.5±6.8a	116.0±50.0a
CON_N+	52.5±14.3a	66.6±14.8a	36.2±6.4a	100.5±45.3a

Reference

Clivot, H., Mouny, J.-C., Duparque, A., Dinh, J.-L., Denoroy, P., Houot, S., Vertès, F., Trochard, R., Bouthier, A., Sagot, S., Mary, B., 2019. Modeling soil organic carbon evolution in long-term arable experiments with AMG model. *Environ. Model. Softw.* 118, 99–113. <https://doi.org/10.1016/j.envsoft.2019.04.004>

Résumé étendu en français

Résumé long

Pour maintenir les rendements des cultures et répondre aux besoins alimentaires en constante évolution d'une population croissante, l'utilisation d'engrais est devenue de plus en plus essentielle. Cependant, la production d'engrais azotés consomme de grandes quantités d'énergie, tandis que les engrais phosphatés et potassiques dépendent de ressources minières non renouvelables. De plus, une utilisation excessive d'engrais minéraux peut entraîner divers problèmes environnementaux et de santé. Il est donc essentiel de trouver des méthodes alternatives pour maintenir les rendements des cultures sans dépendre fortement des engrais minéraux. Parmi les nombreuses alternatives figure le recyclage des produits résiduels organiques (PRO). Les PRO correspondent à l'ensemble des matières organiques produites par diverses activités (agricoles, urbaines, industrielles) et non directement produites sur la parcelle sur laquelle ils sont épandus. Ils comprennent les effluents d'élevage, les composts urbains, les digestats de méthanisation, etc. En augmentant la teneur en matière organique du sol, en améliorant ses propriétés physiques et en augmentant l'abondance et l'activité des micro-organismes, l'application de PRO peut augmenter durablement les rendements des cultures. Les essais au champ de longue durée (LTE) sont considérés comme la méthode la plus fiable et pertinente pour évaluer de manière globale l'impact d'applications répétées de PRO, car les effets cumulatifs de l'application de PRO sur les propriétés du sol et les rendements des cultures peuvent durer plusieurs années et peuvent devenir mesurables après plusieurs années seulement. Cependant, de nombreux travaux basés sur des LTE présentent des lacunes, notamment l'utilisation excessive de PRO dans les LTE pour accélérer la réponse du système, le nombre limité de types de PRO étudiés, le nombre limité d'effets étudiés en même temps et la faible mise en avant des déterminants de ces effets, l'absence de comparaison de LTE contrastés entre eux, et l'analyse statistique insuffisante des données expérimentales. Par conséquent, l'objectif principal de cette étude est d'explorer les multiples effets d'applications répétées de PRO sur la fertilité du sol et la production agricole, pour différents types de PRO (composts urbains, boue d'épuration urbaine et fumier en tant que PRO de référence connu) et doses d'applications, dans deux contextes pédoclimatiques contrastés, en se basant sur deux essais au champ de longue durée en France.

Les deux essais au champ de longue durée mobilisés dans cette thèse sont QualiAgro et PROspective. L'expérience PROspective est située à Colmar, dans le nord-est de la France, caractérisée par un climat semi-continental, un sol de type Calcosol et une succession culturale maïs grain – blé tendre – betterave sucrière – orge de printemps. L'étude comprend deux niveaux de supplémentation en N minéral et 5 amendements organiques comprenant des boues d'épuration (SLU), du compost de SLU et de déchets verts (GWS), du compost de biodéchets (BIO), du fumier bovin (FYM), du compost de fumier bovin (FYMC) et un témoin (CON). Les PRO sont appliquées à une dose de 170 kg N/ha tous les deux ans de 2001 à 2013. Dans la partie de l'essai sans apport de N

minéral, les doses de PRO ont été augmentés et du digestat de biodéchets appliqué depuis 2015 pour obtenir des rendements optimaux, tandis qu'aucun changement n'est survenu dans la partie de l'essai avec N minéral. L'expérience QualiAgro est située à Feucherolles, dans le nord de la France, avec un climat océanique, un sol de type Luvisol et initialement une succession maïs grain – blé tendre. L'essai comprend deux niveaux de supplémentation en N (optimal et minimal) et 4 amendements organiques comprenant du compost de déchets solides municipaux (MSW), du compost de biodéchets (BIO), du compost de boues d'épuration et de déchets verts (GWS), du fumier bovin (FYM) et un témoin (CON). Les PRO sont appliquées à un taux d'environ 4 t C/ha tous les deux ans de 1998 à 2013. Depuis 2014, le site est conduit en agriculture biologique (arrêt du N minéral et des phytosanitaires). La stratégie de fertilisation a été ajustée à 2 t C/ha dans l'ancienne partie avec N optimal, tandis que l'application de PRO a été arrêtée dans la partie de l'essai avec N minimal et que des légumineuses ont été introduits dans la rotation.

L'un des plus grands intérêts de l'utilisation des PRO en agriculture provient de ses avantages pour la productivité des cultures. Ainsi, dans le chapitre III, nous avons analysé l'impact de l'application de PRO sur les rendements des cultures à QualiAgro et PROspective. Pour faciliter l'analyse comparative, nous nous sommes concentrés sur deux cultures communes, le blé tendre et le maïs grain, dans les deux sites. Les résultats ont révélé que la substitution partielle de l'azote minéral par les PRO peut maintenir voire augmenter les rendements des cultures à long terme, tandis que la substitution totale est insuffisante pour maintenir le rendement, surtout la deuxième année suivant l'application des PRO étudiés. A QualiAgro, où une plus grande quantité de PRO est appliquée, les rendements des cultures ont augmenté avec les applications répétées au fil du temps. La combinaison de PRO et d'une fertilisation minérale azotée optimale a conduit à des rendements plus élevés de maïs et de blé par rapport au témoin en azote minéral. Par ailleurs, la combinaison de PRO et d'une fertilisation minérale azotée minimale a atteint le même rendement que le témoin en azote minéral après 3 et 6 applications de PRO pour le maïs et le blé, respectivement. Cependant, à PROspective, les rendements des cultures n'ont pas semblé augmenter au fil du temps après des apports répétés de PRO. La substitution partielle d'engrais minéral par les PRO a maintenu les rendements de maïs mais a diminué les rendements de blé, tandis que la substitution totale a conduit à une diminution des rendements de maïs et de blé par rapport au témoin en azote minéral. Par la suite, nous avons utilisé un modèle statistique GMB (gradient boosting model) pour révéler l'influence relative des entrées de nutriments par les PRO et des caractéristiques du sol sur les rendements relatifs des cultures. Nous avons constaté que l'azote total du sol plutôt que l'apport d'azote minéral était le principal moteur du rendement relatif du maïs, tandis que l'apport d'azote minéral était plus critique pour le blé. De plus, un pH du sol compris entre 7,1 et 8,2 était bénéfique pour la croissance du maïs et entre 6,6 et 8,0 pour la croissance du blé. Sur la base de nos résultats, nous concluons que l'utilisation combinée de PRO et d'engrais minéraux à une dose réduite est une meilleure approche pour maintenir les rendements des cultures et améliorer la fertilité du sol.

Compte tenu du fait que nous avons constaté des effets différents des PRO sur les rendements dans les deux sites, nous nous sommes ensuite intéressés aux effets des PRO sur les propriétés du sol. Par conséquent, dans le Chapitre IV, nous avons exploré les changements, les interactions et les facteurs de contrôle de l'application des PRO sur les propriétés physiques, chimiques et biologiques du sol dans les deux sites. Notre étude a confirmé que l'application à long terme des PRO modifiait significativement les propriétés physiques, chimiques et biologiques du sol, les effets dépendant du taux d'application, des types de PRO et de la fertilité initiale du sol. Les effets d'apports répétés de PRO étaient moins prononcés à PROspective où les sols étaient initialement plus fertiles qu'à QualiAgro et où les doses d'apport de PRO sont plus faibles. Dans les deux sites, les changements dans les propriétés biologiques et la stabilité des agrégats étaient moins affectés que les changements de propriétés chimiques. Bien que l'application régulière des PRO puisse être insuffisante pour améliorer la biologie du sol ou la stabilité des agrégats, elle ne semble pas leur nuire. L'insertion des PRO avec d'autres pratiques (par exemple la réduction du labour, les cultures de couverture, la diversité cultivée) pourrait être une stratégie judicieuse pour améliorer la fertilité du sol dans son ensemble sur le long terme. En parallèle, nous avons constaté que l'application des PRO augmentait la disponibilité des éléments traces, sans que des effets nuisibles sur la biologie des sols, ni sur les rendements et la qualité des récoltes ne soient visibles.

Jusqu'à présent, nous avons constaté que l'effet des applications de PRO sur les propriétés du sol dépend fortement du type de sol et des taux d'application, différents entre QualiAgro et PROspective. Cependant, à notre connaissance, il existe peu d'études relatant des résultats d'essais au champ de longue durée menés sur des sols calcaires, en particulier avec l'application de PRO à des doses couramment utilisées par les agriculteurs, et dans des systèmes de culture réels. Ainsi, pour mieux élucider les effets spécifiques de l'application de PRO dans les sols calcaires, le chapitre V évalue comment divers types de PRO utilisés à la dose réglementaire européenne de 170 kg N/ha soutiennent les rendements des cultures et influent sur les dynamiques de C, N, P et K en se basant sur l'essai PROspective. Les résultats ont montré que la substitution partielle d'engrais azoté minéral par les PRO permettait de maintenir le rendement des cultures (sauf le blé) et les concentrations en N, P et K pour les grains des cultures au niveau du témoin avec engrais minéraux. Les différents types de PRO entraînent des économies d'engrais différentes : la boue d'épuration (SLU) économise le plus d'engrais azoté, la boue et le compost de boue (GWS) économisent le plus d'engrais phosphaté, tandis que le fumier bovin (FYM) et le fumier bovin composté (FYMC) économisent le plus d'engrais potassique. Cependant, sauf pour SLU, la substitution complète de l'azote minéral par les PRO a entraîné une réduction de la biomasse récoltée totale, en particulier dans le cas du blé, où la réduction du rendement était la plus significative. De plus, la substitution complète de l'azote minéral par les PRO a considérablement réduit la concentration en N dans le grain de blé, quel que soit le type de PRO. Nous avons également calculé la valeur de remplacement de l'engrais azoté (NFRV) et avons constaté que SLU et le digestat

avaient la plus grande NFRV (respectivement 58 % et 69 %), tandis que le compost de biodéchets (BIO) avait la plus faible NFRV (21 %) parmi tous les types de PRO. L'application répétée de PRO a considérablement augmenté la teneur en carbone organique du sol (à l'exception de SLU). Aux doses réglementaires européennes et avec une restitution des résidus de culture, la contribution des résidus de culture aux stocks de carbone organique du sol est cependant plus élevée que celle des PRO. La dynamique du phosphore Olsen et du potassium échangeable du sol variait considérablement en fonction du PRO considéré, allant d'une augmentation à une diminution pour des apports en excès de P et K. Enfin, le potentiel de lixiviation des nitrates, estimé via les reliquats entrée hiver, n'a pas augmenté avec l'application de PRO à long terme.

En résumé, notre étude a démontré à la fois les avantages potentiels et les limites de l'utilisation des PRO en agriculture. L'impact de l'application de PRO sur les propriétés du sol et le rendement des cultures dépend fortement de divers facteurs, notamment le type de PRO, le taux d'application et les propriétés initiales du sol. Parmi eux, la quantité de PRO appliquée est le facteur le plus important pour déterminer son effet. SLU avait la plus haute valeur de remplacement d'engrais azoté (NFRV), mais son effet sur le carbone du sol était faible. GWS a connu la plus forte augmentation en phosphore du sol, FYM et FYMC ont connu la plus forte augmentation en potassium du sol, et BIO a eu les NFRV les plus. Néanmoins, l'incorporation de PRO dans une gamme plus large de pratiques agricoles durables peut être une stratégie judicieuse pour améliorer la fertilité et la productivité des cultures. Enfin, notre étude met en avant l'intérêt de disposer d'un réseau d'essais au champ de longue durée pour tester différents PRO dans différents contextes.