University of São Paulo
"Luiz de Queiroz" College of Agriculture

Soil quality in the black oat and soybean succession system irrigated with treated slaughterhouse effluent

Lisiane Brichi

Thesis presented to obtain the degree of Doctor in Science. Area: Agricultural System Engineering

Piracicaba
2024

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Biosystem Engineer

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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Advisor:
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DEDICATION

To my family: my parents Marilene and Aureo Brichi, and my brother João Vitor Brichi.

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To my parents, who have always supported me in this relentless pursuit of knowledge, and to my brother, who has consistently provided me with emotional support on this journey.

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To all those who believe in science.

CONTENTS

RESUMO

Qualidade do solo no sistema de sucessão aveia-preta e soja, irrigado com efluente tratado de abatedouro

O aumento na demanda por alimentos tem ocasionado a busca por alternativas que não gerem pressão ambiental e que sejam alinhadas com moldes sustentáveis de agricultura. Neste contexto o reúso de efluente tratado de abatedouro (ETA), se posiciona como uma estratégia ambientalmente e economicamente viável, e socialmente correta. Além disso, apresenta-se como uma excelente alternativa para manutenção da saúde do solo, devido aportar nutrientes e matérias orgânica. Deste modo, os objetivos deste trabalho foram mapear, por meio de avaliações bibliométricas, como este assunto de pesquisa tem sido avaliado nos últimos anos e qual a relevância do tema (capítulo 1), além da avaliação dos impactos deste tipo de efluente em um sistema de sucessão (aveia-preta/soja) nos aspectos voltados a qualidade/saúde do solo (capítulos 2, 3 e 4). Para tanto, o experimento foi delineado em blocos casualizados, com cinco tratamentos e quatro repetições, a saber: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50% e T5 - 25% das doses de nitrogênio (N) recomendadas para as culturas por meio de irrigação com ETA. No tratamento T1 - 0% ETA (testemunha), a dose necessária de N foi fornecida por meio de fertilizante nitrogenado, na forma de ureia. Para a comparação dos indicadores de qualidade do solo, um sexto tratamento, T6 - NV, foi estudado, correspondendo a uma área de vegetação nativa (floresta semidecídua sazonal). Os resultados do primeiro capítulo demonstram que, embora não muito frequentemente estudado, o ETA pode impactar positivamente a qualidade química do solo e que os demais indicadores relacionados a qualidade física e biológica do solo necessitam de mais estudos. Já os resultados dos capítulos 2 e 3, demonstraram que a dose de 75% ETA mostra-se adequada as características físicas e químicas do solo. Porém, no capítulo 4 não houve evidências que as diferentes doses de ETA impactaram os índices estudados de qualidade do solo. Importante ressaltar que a aplicação de ETA não foi prejudicial do ponto de vista da qualidade do solo, o que indica o potencial de reuso deste efluente. Espera-se que o presente estudo incentive pesquisas futuras para que cada vez mais medidas como essa, diretamente conectadas com o conceito de economia circular, sejam realizadas.

Palavras-chave: Saúde do solo, Reúso de água, Sucessão de culturas, Indicadores

ABSTRACT

Soil quality in the black oat and soybean succession system irrigated with treated slaughterhouse effluent

The increasing demand for food has led to the search for alternatives that do not generate environmental pressure and align with sustainable agricultural practices. In this context, the reuse of treated slaughterhouse effluent (TSE) emerges as an environmentally and economically viable, as well as socially responsible strategy. Additionally, it presents an excellent option for the restoration of soil health due to its high nutrient content and organic matter. Thus, the objectives of this thesis were to map, through bibliometric evaluations, how this research topic has been assessed in recent years and the relevance of the subject (Chapter 1), as well as to evaluate the impacts of this type of effluent on a crop succession system (black oats/soybean) concerning soil quality/health aspects (Chapters 2, 3, and 4). To accomplish this, the experiment was designed in randomized blocks, with five treatments and four replications, namely: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50%, and T5 - 25% of the recommended nitrogen (N) doses for crops through TSE irrigation. In treatment T1 - 0%TSE, the required N doses were supplied using nitrogen fertilizer in the form of urea, through sprinkler irrigation. For the comparison of soil quality indicators, a sixth treatment, T6 - NV, was studied, corresponding to an area of native vegetation (seasonal semideciduous forest). The results from the first chapter demonstrate that although not extensively studied, TSE can positively impact soil chemical quality, and other indicators related to soil physical and biological quality require further investigation. Meanwhile, the results from Chapters 2 and 3 showed that the 75% TSE dose (T3) appears suitable concerning soil physical and chemical quality. However, in Chapter 4, there was no evidence that different doses of TSE impacted the soil quality indices studied. It is essential to highlight that the application of TSE was not detrimental to soil quality, indicating the potential for effluent reuse. It is hoped that this study will inspire future research to conduct more measures like this one, directly aligned with the concept of a circular economy.

Keywords: Soil health, Water reuse, Crop succession, Indicators

1. GENERAL INTRODUCTION

The demand for water resources has increased by about 1% annually due to population growth, and consequently, the volume of effluents generated has also increased (WWAP, 2018; Abegurin et al. 2016; Darvishi et al., 2010). In this context, many are the sectors that compete for water, gaining prominence in the irrigation of crops, responsible for the withdrawal of about 70% of all available water in the world (WWAP, 2017). In Brazil, it is estimated that 8.5 million hectares (Mha) are irrigated, with the southeast region being the most expressive (ANA, 2021). For this reason, the application of wastewater in agriculture has presented strong adherence worldwide by providing nutrients, reducing the need for synthetic fertilizers, and constituting an environmentally friendly solution (Al-Hamaiedeh; Bino, 2010; Fito; Van-Hulle, 2020; Helmecke et al., 2020).

Both in Brazil and worldwide, the animal slaughtering and meat processing industries are among the most polluting in terms of total amounts of effluent generated, as well as in terms of their characteristics, since they demand large amounts of water in their processes (Rahman et al., 2014; Ribeiro, 2013; Harvey et al., 2017). In general, this type of effluent presents high biochemical oxygen demand (COD), high concentrations of organic matter, nitrogen, phosphorus, total suspended solids (TSS), and salts, in addition to substances such as ammonia, potentially toxic metals (MPT) and the presence of pathogens (Bustillo-Lecompte; Mehrvar, 2015; Harris et al., 2015). Such characteristics and their concentrations may vary between countries, due to the treatment steps required by legislation, and among the species of animals slaughtered/processed, since water volumes in the slaughter process may differ (Liu; Haynes, 2011a; Harris et al. 2015; Rahman et al., 2014).

The incorrect disposal of these effluents can negatively impact the quality of the water and soil and for this reason requires adequate treatment, either for disposal into water bodies or for agricultural reuse. There are three stages of wastewater treatment: primary, aiming at the removal of coarse solids (grading and desanding); secondary, aiming at the removal of organic matter and dissolved solids (anaerobic/aerobic reactors and decanters), and tertiary, to meet the required release standards (disinfection) (Liu; Haynes, 2011a).

In the national context, Brazil released Resolution Conama 503 on December 14th, 2021, about wastewater regulations. This resolution delineates guidelines for the utilization of wastewater in fertigation systems, specifically targeting effluents from the food, beverage, dairy industries, slaughterhouses, and rendering plants. The resolution sets forth limits for E. coli in agro-industrial effluents used for irrigating food crops where the edible portion comes into contact with the soil. Additionally, it introduces parameters for monitoring and characterizing soils pre- and post-application of agro-industrial effluents. These include assessments of pH, electrical conductivity, organic matter, P, K, Ca, Mg, Al, S, Na, B, Cu, Fe, Zn, Mn, H+ Al, soil texture, and soil water infiltration.

To minimize the impacts of the incorrect disposal of this type of effluent, secondary treatment (biological) linked to agricultural reuse is a viable alternative for recycling water and minimizing fertilizer costs (Menegassi et al., 2020; Vergine et al., 2017). However, to measure the real benefits of this practice, soil quality must be evaluated, since its correct maintenance leads to high levels of productivity (Bünemann et al., 2018; Guo et al., 2017). According to the U.S. Natural Resources Conservation Service, “soil health, also referred to as soil quality, is defined as the ongoing ability of soil to function as a vital living ecosystem that supports plants, animals, and humans.” Soil quality/health depends on intrinsic factors and management-sensitive factors and can be assessed by analytical methods or visual methods (Buneman et al., 2018; Andrews et al., 2004; Shepherd et al., 2008). Furthermore, selected SQ indicators must be measurable, sensitive to management changes, and interpretable (Doran and Parkin, 1996; Buneman et al., 2018).

Guo and Sims (2003) cite that, in general, irrigation with effluent can alter several physicochemical soil properties such as infiltration, hydraulic conductivity, fertility, density, porosity, and pH. In addition to these influences, Becerra-Castro et al. (2015), citing that the modification of soil physicochemical parameters due to wastewater irrigation can induce changes in microbial communities. Several studies have shown that irrigation with wastewater from slaughterhouses can raise the content of organic matter, nutrients, pH, and activity/diversity of microbial communities, however, can lead to problems linked to salinity and sodicity of soils (Menegassi et al, 2020; Alabi et al. 2019; Shilp et al. 2018; Matheyarasu et al. 2017; Oliveira et al. 2017; Abegurin et al., 2016; Liu; Haynes, 2013; Silva-Neto et al., 2013).

Thus, knowing the potential benefits of agricultural reuse of treated slaughterhouse effluent on soil health, the objective of this work was to evaluate the quality (chemical, physical and biological) of the soil in a black oat - soybean succession system, irrigated with treated effluent of a slaughterhouse. To this end, the specific objectives were: the bibliographic mapping of the topic, selection of representative indicators and accurate methodologies for the correct understanding of the impacts of the TSE on the physical, chemical and biological quality of the soil.

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2. SLAUGHTERHOUSE EFFLUENTS AND SOIL QUALITY: A REVIEW

Abstract

The increased demand for food and water in a growing population leads to the need for sustainable food security strategies, in which the reuse of agro-industrial effluents may assist this challenge. However, only a few studies aimed to study the impact of irrigation with slaughterhouse effluents on soil quality. Thus, this study aims to gather, classify, analyze and discuss bibliometric information as well as information about soil quality assessments in works about irrigation with slaughterhouse effluent. Bibliography research was conducted on the Web of Science and Scopus databases and a total of 29 records were selected and analyzed. Bibliometric aspects were evaluated and information about the methodological description of the experiment, the effluent and about soil quality was also extracted from the database. Australia, New Zealand, Brazil and Nigeria are the countries with the most publications from 1970 to 2022, with the first two countries having scientific cooperation with each other. These studies assessed mainly chemical soil properties, from which total/available N, total/available P and exchangeable K the ones that most positively affected soil quality. Soil physical and biological indicators of soil quality were poorly investigated and therefore further research is needed, since they contribute to the correct understanding of soil health and strategic decision-making aimed at maintaining crop productivity and ecosystem services provided by the soil.

Keywords: soil health, wastewater reuse, agro-industrial wastewater, scientific production, sustainability.

2.1 Introduction

Water demand has increased about 1% annually due to population growth, leading to an increase in the volume of effluents generated (Abegunrin et al., 2016a; Darvishi et al., 2010; WWPA, 2018). In this framework, many sectors compete for water resources, with emphasis on crop irrigation, which accounts for 70% of water use worldwide (WWAP, 2017). Wastewater application in crop fields rises as an alternative to decrease freshwater consumption in agriculture, providing nutrients, reducing the need for synthetic fertilizer, and constituting an environmentally friendly solution (Fito and Van Hulle, 2020; Helmecke et al., 2020).

Slaughterhouses and meat processing industries demand high amounts of water in their processes, which includes slaughtering and cleaning, in which the resulting wastewater has potentially polluting characteristics (Harvey et al., 2017; Liu and Haynes, 2011; Rahman et al., 2014; Ribeiro et al., 2013). Typically, such effluents have high concentrations for biochemical oxygen demand (BOD), organic matter, nitrogen, phosphorus, total suspended solids (TSS) and salts, in addition to substances such as ammonia, potentially toxic metals (PTM) and pathogens (Bustillo-Lecompte and Mehrvar, 2017; Harris and McCabe, 2015). The exact content for effluents from slaughterhouses and meat processing industries may vary between countries, as well as depending on animal species and on the quantity of animals slaughtered/processed (Harris and McCabe, 2015; Liu and Haynes, 2011; Musa et al., 2018; Rahman et al., 2014). Therefore, either for disposal in water bodies or for agricultural use, the generated effluents need adequate treatment, wherein the anaerobic treatment is one of the most recommended methods (Bustillo-Lecompte et al., 2016; Bustillo-Lecompte and Mehrvar, 2017, 2015). According to Menegassi et al. (2020) and Vergine et al. (2017), the biological treatment linked to agricultural reuse constitutes a viable alternative for recycling water and minimizing costs with fertilizers. For this reason, the establishment of standards, guidelines and legislation applied to agricultural reuse are extremely important, and in this context, the guidelines on water quality for agricultural purposes by the World Health Organization (WHO) and by the United States Environmental Protection Agency (USEPA) are extremely important (WHO, 1989; USEPA, 2012).

In order to measure the benefits of wastewater reuse, it is of paramount importance to monitor soil quality (soil health), since a proper soil management promotes the functionality of ecosystem services provided by the soil and improve food security, a result of the satisfactory crop yields (Bünemann et al., 2018; Hurni et al., 2015; McBratney et al., 2014). Soil quality is defined as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997).

Several studies have shown that wastewater irrigation may change soil physical, chemical and biological conditions, including an improvement in soil fertility, although it may also lead to salinization and sodification (Lal, 2009; Sandri e Rosa, 2017;Abd-Elwahed, 2018). Soil organic matter is often cited as the soil quality indicator most influenced by wastewater irrigation, which leads to a higher soil organic matter content (Abd-Elwahed, 2018; Becerra-Castro et al., 2015; Sánchez–González et al., 2017). However, there is a lack of studies about wastewater irrigation specifically with slaughterhouse effluent and its impact on soil quality. The few studies about that relate an increase in soil fertility and CEC, changes in soil pH, increase in soil salinity and sodicity, as well as a reduction in soil bulk density in the topsoil (Guo and Sims, 2003a; Matheyarasu et al., 2016a; Menegassi et al., 2020; Osemwota, 2010a).

Therefore, monitoring the published literature about irrigation with slaughterhouse effluent and its impact on soil quality is of primary importance to comprehensively understand the state of the art about this topic. Data about institutions, countries, authors and other bibliometric parameters obtained through systematic review techniques would be useful for information mapping and management, contributing to scientific advances about this topic. Thus, this study aims to gather, classify, analyze and discuss bibliometric information as well as information about soil quality assessments in works about irrigation with slaughterhouse effluent.

2.2 Material and Methods

The bibliographic search to build the bibliometric review was performed by combining search terms and consulting the databases Web of Science (https://www.webofscience.com/) and Scopus (https://www.scopus.com/). The search was performed by selecting the option "topic" in both databases, which considers title, abstract and keywords. The searching terms were combined using Boolean operators as follows: TOPIC: ("slaughterhouse" OR "meat" OR "abattoir" OR "meatworks" OR "meat industry" OR "meat processing factory") AND TOPIC: ("effluent" OR "wastewater") AND TOPIC: ("soil quality" OR "soil health" OR "soil properties" OR "soil attributes" OR "soil " OR " soil nutrient*" OR "soil fertility"). The search led to 100 records in WOS and 127 in Scopus, which were retrieved on February 7th, 2022, and filtered to include only articles in English. All possible publication years were considered. The steps related to searching and sorting the articles were summarized in Figure 1.

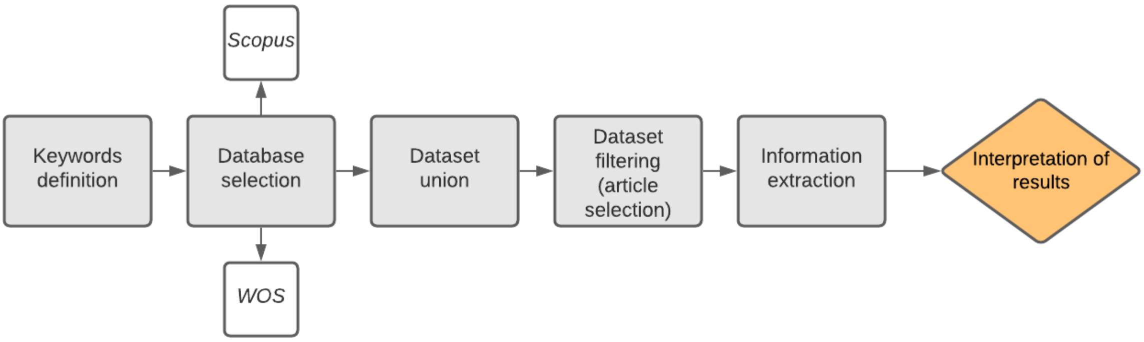

Figure 1. Steps related to selecting, sorting and interpreting articles and the associated information within the database.

The first step for data triage was to combine records from both databases and to merge duplicates. In order to do so, the R package "bibliometrix" was used in the RStudio® environment, which resulted in an output file containing 156 records and their associated information in the xlsx format. Thereafter, dataset filtering (article selection) was performed following two criteria: only records that studied slaughterhouse effluent and concurrently assessed the impact of the effluent application on any physical, chemical or biological soil property. This step was completed by three reviewers and resulted in a total of 29 records selected. Although a higher number of records were found after database union, many of them were not considered because: a) there was no assessment of soil quality indicators, b) there was no slaughterhouse effluent, or c) there was no data related to chemical and physical characterization of the applied effluent.

After article selection, the following bibliometric data was extracted from each record: publication year, citation rate by year, number of records by journal, number of records by institution, keyword co-occurrence, number of records by country, and co-authorship between countries. The resulting data was processed by using the software's Microsoft Office Excel (version 2019) and VOSviewer (version 1.6.18), in which the latter was used for bibliometric mapping considering the data for co-authorship, co-citations, citations, keyword co-occurrence and bibliographic coupling (van Eck and Waltman, 2010).

In addition to the bibliometric data, information about the methodological description of the experiment, the effluent and about soil quality was also extracted from the database. This includes the experimental conditions, physico-chemical properties of the effluent, methods of effluent treatment, crops irrigated with slaughterhouse effluent, soil order, soil texture, soil depth, soil chemical, physical, and biological indicators of soil quality assessed, as well as the impact of irrigation with slaughterhouse effluent on soil quality.

2.3 Results and Discussion

2.3.1 Bibliometric data: publication years, authors, journals and institutions

The first paper about irrigation with slaughterhouse effluent and its impact on soil quality was published by Wells and Whitton (1970). It is important to mention that all possible publication years were considered in this search, and therefore the earliest record is from 1970. A total of 29 articles were published up to 2022, with an average of less than an article per year (0.6) (Figure 2). The year with the highest number of articles was 2017, in which four papers were published, including Oliveira et al. (2017a), Oliveira et al. (2017b), Luchese et al. (2017) and Matheyarasu et al. (2017), as described in Supplementary Table 1 (Appendix A).

Although wastewater reuse in agriculture is a growing concern due to factors such as shortage of drinking water, population growth, climate change and agricultural expansion (Becerra-Castro et al., 2015), research related to impacts of reusing slaughterhouse effluent on soil quality is still poorly investigated. This might be related to the imposed challenge of recycling slaughterhouse wastewater due to local legal sanitary requirements as well as challenges in technical implementation (Philipp et al., 2021).

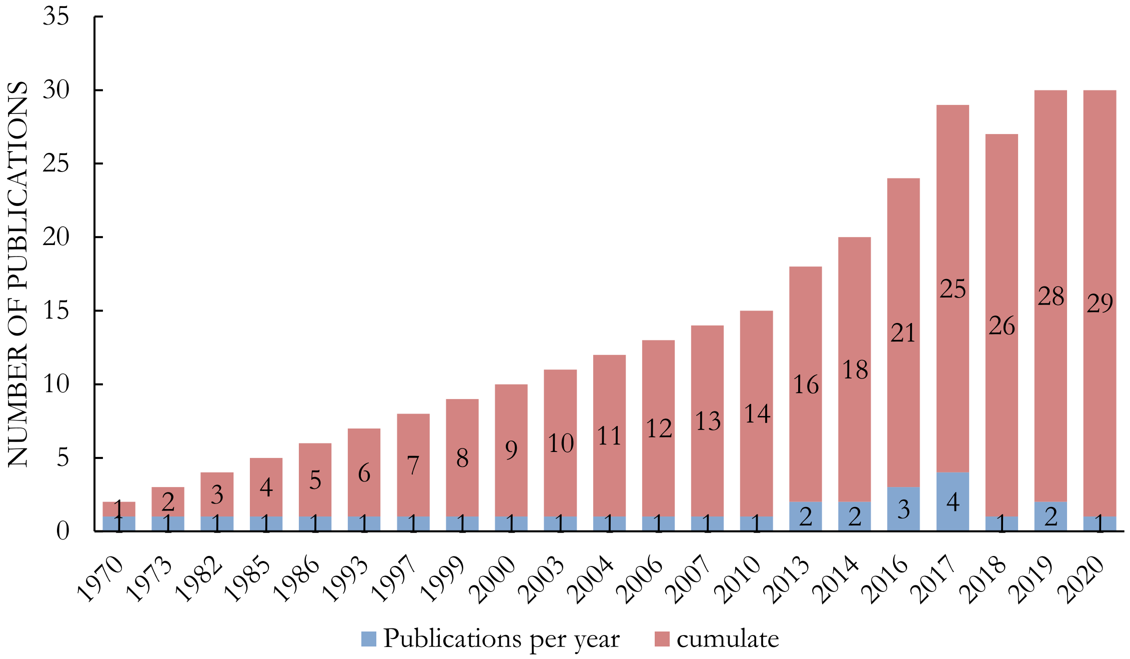

Figure 2. Number of publications by year related to studies about irrigation with slaughterhouse effluent and its impact on soil properties (n = 29).

The most cited article is the one from Abegunrin et al. (2016b) which assessed soil-plant systems with three types of wastewaters (cassava effluent, abattoir and bathroom wastewater) and two crop species (eggplant and spinach) (Figure 3). The high impact of this article relies on the great potential of effluent reuse, especially from slaughterhouses, as its application boosts soil fertility and enhances crop yield, although there might be risks associated with it, such as soil salinization and sodification. Other risks and opportunities, such as an increment in nitrous oxide (N2O) emission and recovery of soil total nitrogen, are also reported in the most-cited publications (Bhandral et al., 2007; Guo and Sims, 2000).

The work of Menegassi et al. (2020), which was cited nine times until the moment of bibliographical searches, is of great importance. The authors report the impacts of applying slaughterhouse effluent on pasture (coast-cross) growth, soil fertility and mention the possibility of a complete replacement of nitrogen fertilization due to high concentrations of nitrogen in the effluent. Therefore, it is notable that this research topic is of great importance and hence soil quality assessments in areas where agro-industrial effluents are applied should be further studied, especially areas with slaughterhouse effluent.

Regarding the number of records by journal, there is no clear trend and the papers are well-distributed between journals (Figure 4). However, it is important to mention that the journals "Bioresource Technology", "New Zealand Journal of Agricultural Research" and "Water, Air, and Soil Pollution" have a total of three published papers each. Nevertheless, the most cited paper (Abegunrin et al., 2016) was published in "Catena".

Institutions from Australia and New Zealand are the most prominent in terms of funding studies about irrigation with slaughterhouse effluent and its impact on soil quality (eg. University of South Australia). Furthermore, 23 institutions were listed within the 29 papers studied in this review (please see supplemental material 1 – Appendix A), suggesting that there is no leading institution studying this topic.

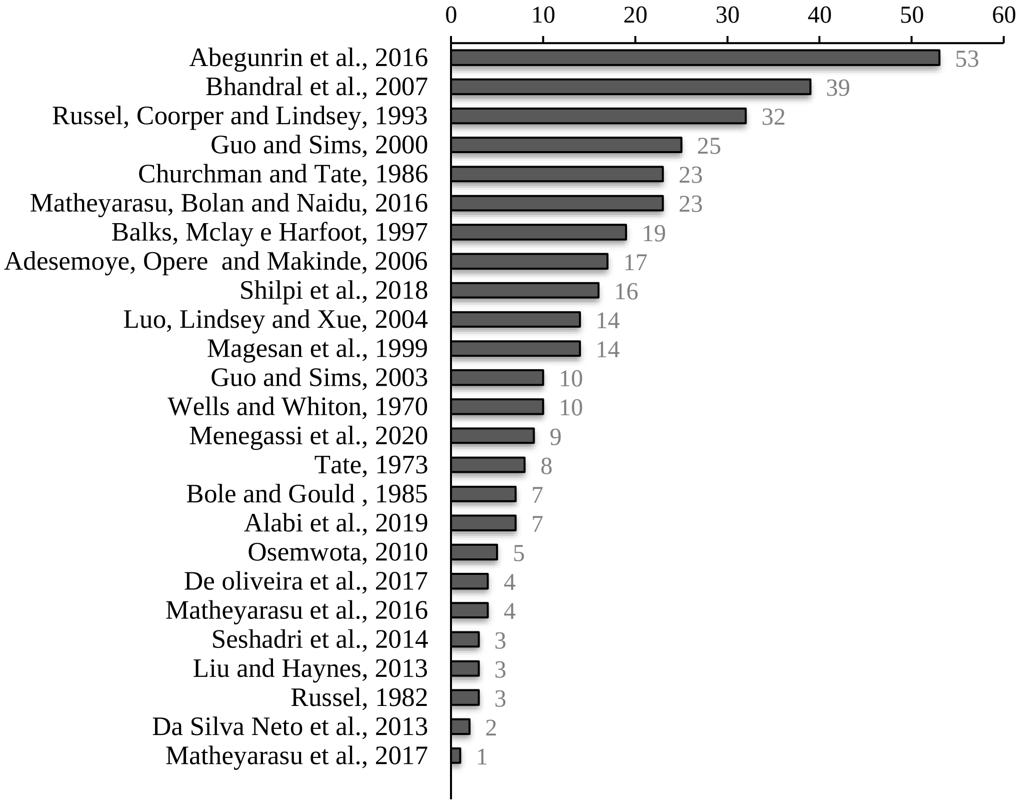

Figure 3. Number of citations within the studied database (n=25). Records without citations were not considered.

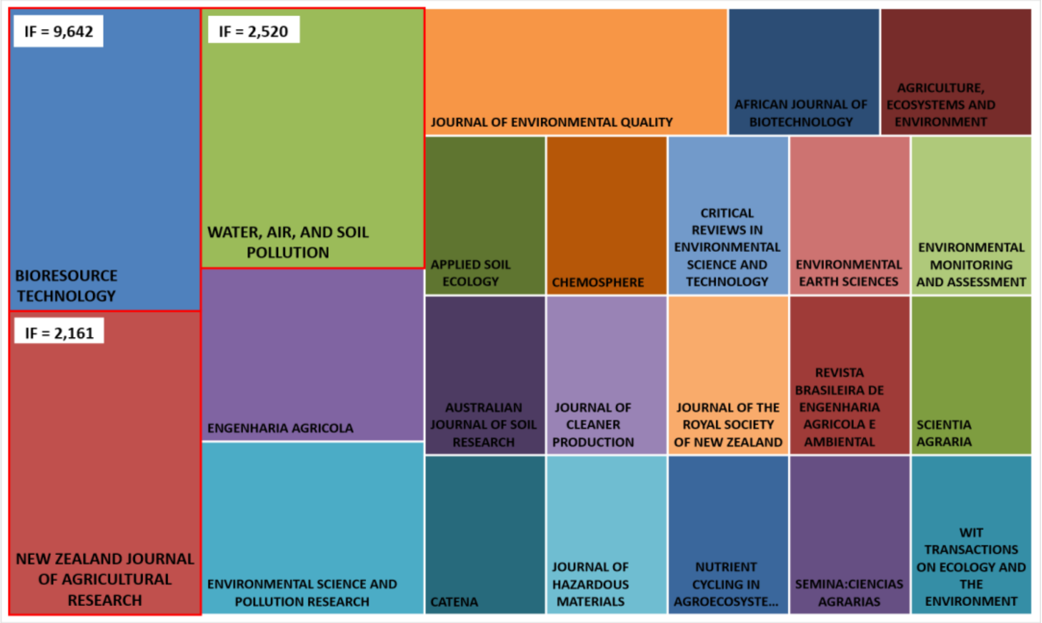

Figure 4. Number of publications by journal within both Scopus and Web of Science (IF = Impact Factor).

By analyzing the bibliographic coupling, which assess the similarity between records from their cited references (Lucas and Garcia-zorita, 2014; Thelwall and Wilkinson, 2004) it is possible to identify that only 8 out of the 29 papers share citations. Records that share citations are from the same authors, and the highest number of shared articles is three, between papers from Oliveira et al. (2017a) and Oliveira et al. (2017b) (Figure 5). Such outcome suggest a lack of connection between centers that study irrigation with slaughterhouse effluent and its impact on soil quality.

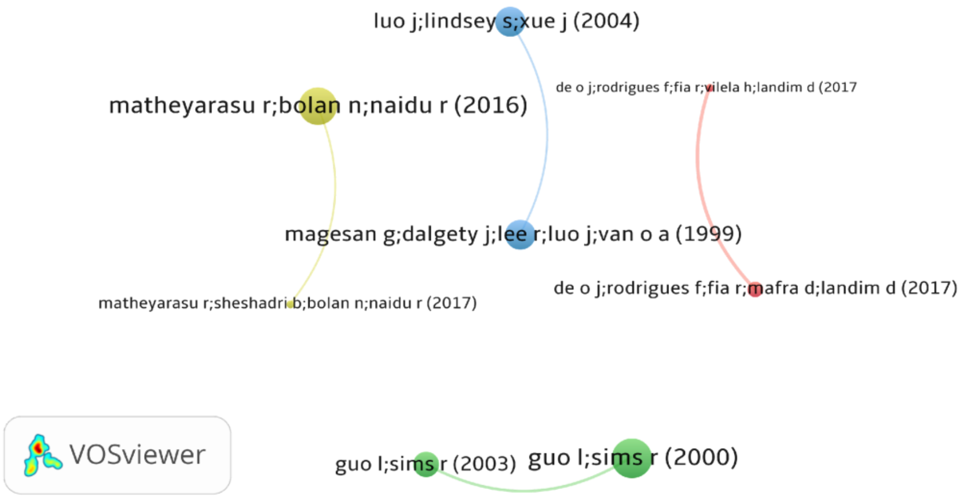

Figure 5. Map of bibliographic coupling from records with bibliographic connections between them (n=8). The size of a circle is related to the number of works cited in a paper and the line weight connecting circles is related to the number of citations shared between papers.

The co-occurrence of keywords analyzes the frequency and connection between them in the considered database in order to find main topics studied and the direction in which research is going (Radhakrishnan et al., 2017; Zhang et al., 2012). From the 29 records reviewed in this work, 398 keywords were retrieved. From this total, considering those with at least four occurrences, two clusters can be noted from the co-occurrence map (Figure 6). The terms “soil”, “irrigation”, “wastewater”, “soil properties", “effluent", “nitrogen” and “slaughterhouse” occurred with the highest frequency and connection, in which the term “soil” is placed in the central portion of the map. The green cluster consists of terms related to soil properties and their connection with effluent, while the red clust is connected to terms related to irrigation and wastewater treatments from slaughterhouses.

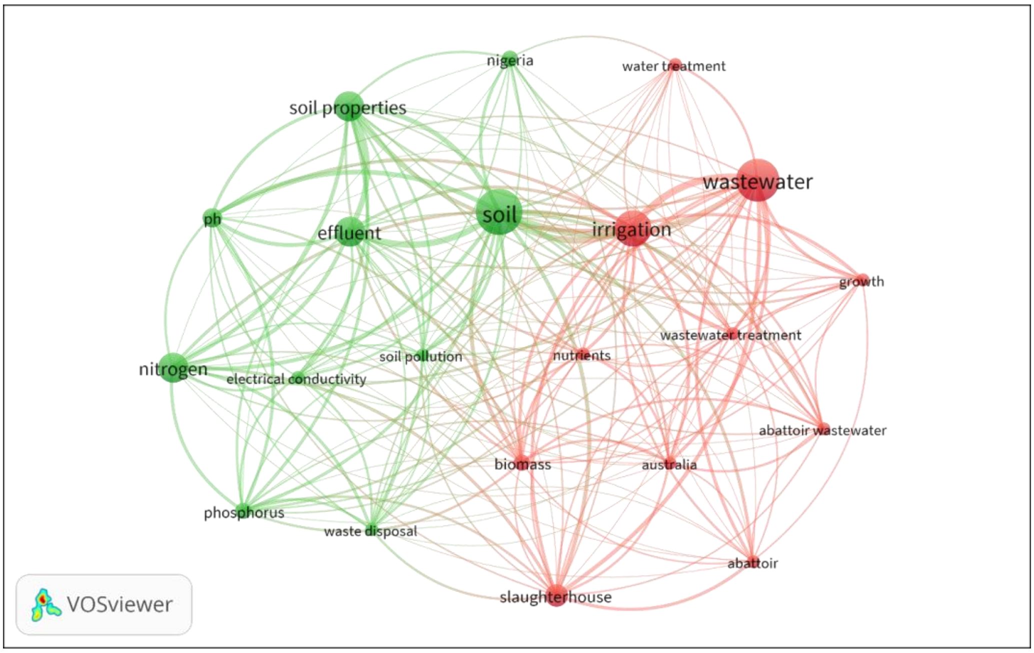

Figure 6. Map of co-occurrence of keywords, considering a minimum frequency equal to four. The size of a circle is proportional to the number of citations in records and the line weight is related to the number of connections between keywords.

2.3.2 Countries and co-authorship

New Zealand and Australia are the countries with the most published articles in this review's dataset (31.03 and 24.14, respectively), working together with institutions from the United Kingdom, Canada and South Korea. Brazil (20.69% of publications) and Nigeria (17.24%) are also main publishing countries, although not as much connected with other countries, as Brazil is only connected with Spain (Figure 8).

All cited countries, except Nigeria, have made significant contributions over the years about water reuse in agriculture. A quick survey combining the terms "wastewater reuse" OR "irrigation AND "effluent" AND "soil", result in 1659 results in WOS (web of science), where Australia, New Zealand and Brazil are ranked in 3rd, 5th and 7th in terms of number of records (146, 130 and 114, respectively). Similar results can be found in Scopus (Figure 7). Most of these records assess the impact reusing treated domestic sewage on soil, crops and groundwater (Arienzo et al., 2009; Duarte et al., 2008; Urbano et al., 2017). Although only 29 records assessed these same parameters with slaughterhouse effluence, they follow the same trend from studies for treated domestic sewage.

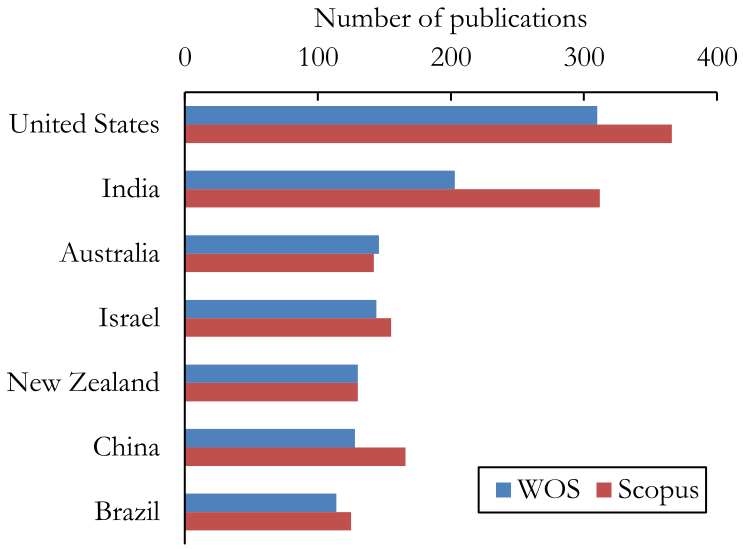

Figure 7. Number of publications related to agricultural reuse of wastewater and soil quality from the seven most relevant countries in this research area (United States, India, Australia, Israel, New Zealand, China and Brazil) since 1970.

Brazil and Australia are important beef producers and exporters, and thus both countries produce great amounts of slaughterhouse effluents (Bustillo-Lecompte and Mehrvar, 2017; Haselroth et al., 2021; Matheyarasu et al., 2016b). In 2021, beef production in Brazil and Australia was 9 and 2 million tonnes, respectively ((FAO, 2021)). In addition, the quantity of water used for pasture irrigation and the demand for fertilizers also follow the growth of the agricultural sector for both countries (Matheyarasu et al., 2017; Menegassi et al., 2020). Therefore, there is a great opportunity for wastewater reuse in these countries. Similarly, irrigated areas in New Zealand increased about 90% in the last 15 years (Graham et al., 2022), and traditionally, irrigation with slaughterhouse effluent is allowed and usual in the country (Guo and Sims, 2003; Luo et al., 2004; Speir, 2002).

Although Nigeria is not worldwide known for its agricultural sector, the arid climate in some regions leads to water shortages, directly affecting agriculture. Thus, wastewater reuse is of primary importance, although there is no legislation about using agricultural reuse and its impact on soil quality in Nigeria (Abegunrin et al., 2016).

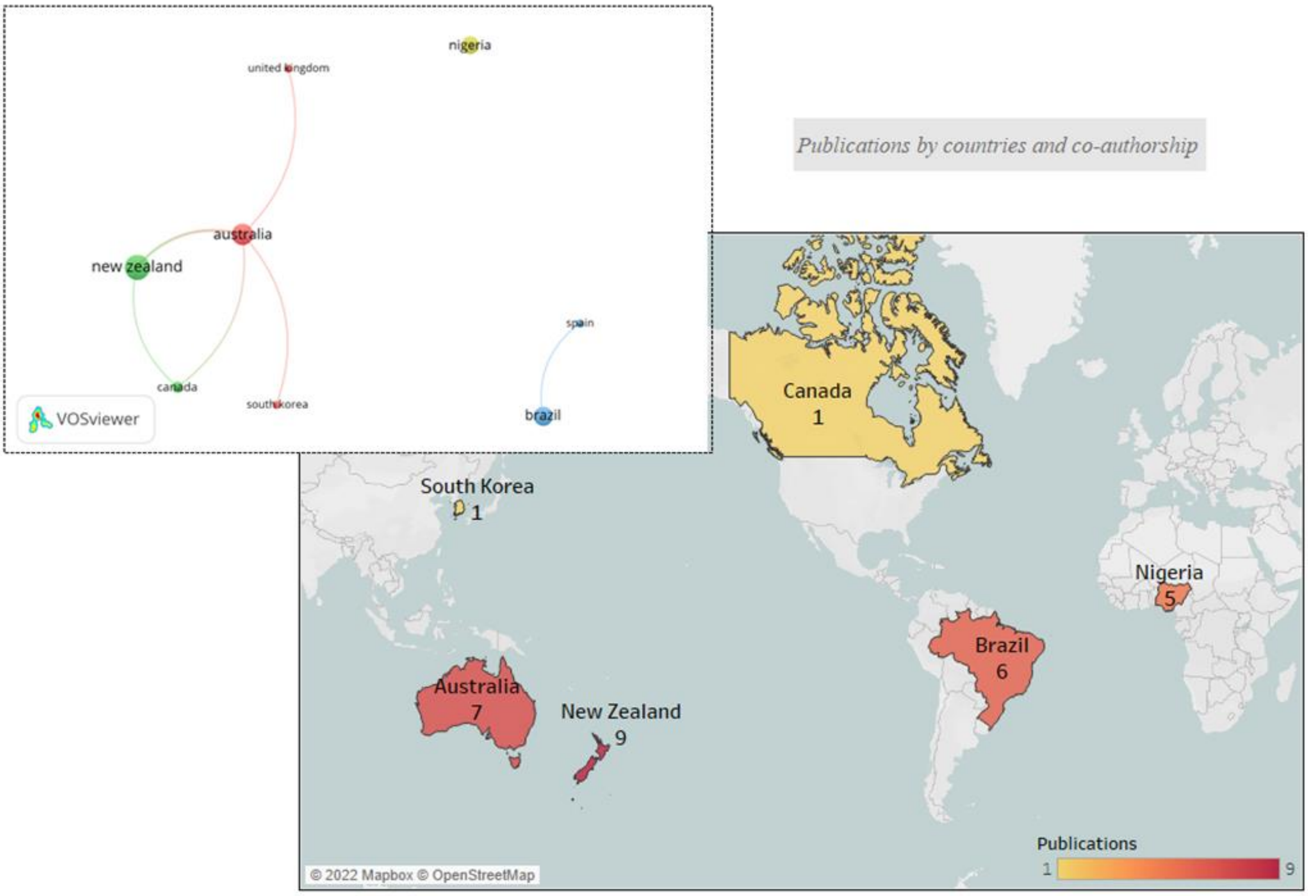

Figure 8. Bibliometric map for co-authorship between countries and the number of publications by country. The size of a circle is proportional to the number of publications by country and the line weight is related to collaborations between countries.

2.3.3 Experiment type, effluent treatment and cultivated crop

The reviewed articles had different methodological conditions to study the impact the application of slaughterhouse effluent on soil quality. From the 29 works, 15 had experimental plots in field experiments, six were carried out in pots, three in soil columns, and five in areas where the effluent was applied but there was no experimental plot (please see supplementary table 2). Most publications reported physico-chemical properties of the applied effluent (please see supplementary table 3 – Appendix A), without mentioning the irrigation method used to apply slaughterhouse effluent, only citing the total water depth.

Regarding the effluent treatment, most publications reported biological treatment (41.38%), followed by preliminary treatment (31.03%), no treatment mentioned (24.14%) and other types of treatments (10.34%) (Figure 9). Biological treatment, which can be divided into aerobic and anaerobic system (Bustillo-Lecompte and Mehrvar, 2015) is considered the most suitable for treating slaughterhouse effluent due to its high capacity to remove organic matter and nutrients (Mittal, 2006; Matheyarasu et al., 2015). Furthermore, anaerobic systems are preferred as they have a higher treatment efficiency, a lower complexity, lower sludge production and the possibility of producing biogas (methane) (Bustillo-Lecompte and Mehrvar, 2017; Liu and Haynes, 2011).

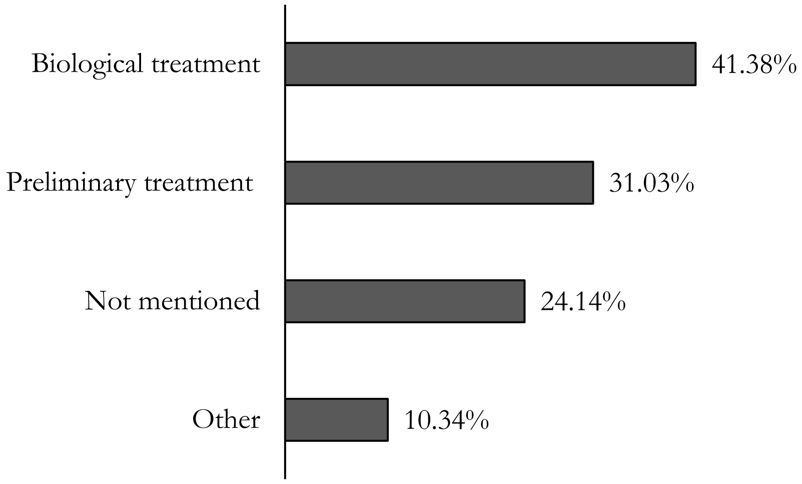

Figure 9. Frequency of treatment type used in the slaughterhouse effluent from 29 reviewed publications. *Others: physico-chemical treatments, direct application into the soil and solution with moringa seeds.

In relation to crops, most works assessed the application of slaughterhouse effluent on pasture (34.48%) and forages (27.59%), suggesting a great potential for reuse in crops grown for animal feed (Figure 10). In addition, 24.14% of works did not assess any plant species.

The result of most works assessing the impact of applying slaughterhouse effluent on crops intended for animal and non-human food might be related to the lack of legislation. Although research about agricultural reuse has gained attention due to climate change and water shortages, the associated legislation is still poorly defined. The guidelines suggested by the World Health Organization (WHO) and by the United States Environmental Protection Agency (USEPA) are of primary importance, as they provide reference values for water quality assessments (USEPA, 2012; WHO, 1989). However, most guidelines warn about the risk of bacterial contamination when wastewater is used in food crops (Jeong et al., 2016).

Considering legislation about wastewater, Brazil issued the resolution Conama 503 on December 14th, 2021, which "defines criteria and procedures for wastewater reuse in fertigation systems with effluents from food, beverage and dairy industries, slaughterhouses and rendering plants". As Brazil is one of the main countries studying soil quality in soils irrigated with slaughterhouse effluent, this recent resolution may create opportunities for wastewater reuse in other crops rather than pastures. The resolution establishes tolerance limits for E. coli in agro-industrial effluents for irrigated food crops in which the edible part is in contact with the soil. Another important contribution is the establishment of criteria/parameters to monitor and characterize soils before and after the application of agro-industrial effluents, such as pH, electrical conductivity, organic matter, P, K, Ca, Mg, Al, S, Na, B, Cu, Fe, Zn, Mn, H+ Al, soil texture and soil water infiltration.

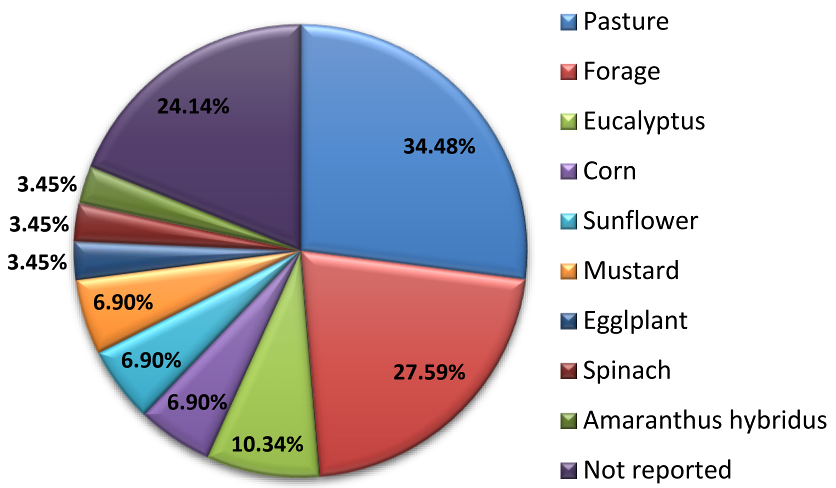

Figure 10. Frequency of crops assessed in publications related to the application of slaughterhouse effluent and soil quality (n=29).

2.3.4 Soil classification and soil quality indicators

18 out of the 29 reviewed papers mentioned the soil order (Soil Survey Staff, 1999), which led to 11 papers (38% of papers) without soil classification. Within the 18 papers, 20 soils were classified in the following orders: Andisols (30%), Inceptisols (20%), Oxisols (20%), Alfisols (10%), Entisols (10%), Mollisols (5%) and Vertisols (5%). All Andisols were located in New Zealand and had either silt loam or sandy loam texture. Although Andisols only cover 1.8% of land mass they have been used for generations, and conservation practices have been installed in most situations (Eswaran and Reich, 2005). Similarly, to Andisols, all Incepitsols (ref) studied were located in New Zealand. Oxisols, contrairly, were located in Brazil and all of them were clayey soils, which is commonly aggregated in a strong grade of fine and very fine granular structure (Eswaran and Reich, 2004), leading to a rapid permeability.

Regarding soil texture in the reviewed database, 31.03% of papers did not mention it (Adesemoye et al., 2006; Araujo et al., 2019; Bhandral et al., 2007; Bole and Gould, 1985; Liu and Haynes, 2013; Matheyarasu et al., 2017, 2016a, 2016b; Shilpi et al., 2018), 27.59 % studied sandy loam soils (Abegunrin et al., 2016c; Arku and Musa, 2014; Guo and Sims, 2003b, 2000; Russell, 1982; Russell et al., 1993; B Seshadri et al., 2014; N Wells and Whitton, 1970), 13.79% silt loam (Balks et al., 1997; Churchman and ate, 1986; Luo et al., 2004; Magesan et al., 1999), 10.34 % clay (Menegassi et al., 2020; Oliveira et al., 2017a; Oliveira et al., 2017b), 10.34% sand (Alabi et al., 2019; da Silva Neto et al., 2013; Osemwota, 2010b), 3.45% clay loam (A. V Luchese et al., 2017) and 3.45% loamy sand (Russell, 1982).

The work of Oliveira et al. (2017b) investigated leaching through soil columns of a clayey Oxisol and found a significant nitrate leaching when wastewater from a swine slaughterhouse was applied. Similarly, Matheyarasu et al.(2016c) mentioned nitrate leaching in groundwater as a potential water pollutant, and they suggest nitrogenous inhibitors and efficient farm budgeting for better nitrogen management and to contribute to a more sustainable agriculture. Seshadri et al. (2014) recommended the use of flyash and redmud as alkaline industrial by-products to reduce P leaching after studying columns of sandy soils irrigated with slaughterhouse wastewater.

In relation to studied soil depth, wihin the 15 from experimental plots in fields, 53,33% assessed soil properties between 0 - 20 cm, and 33,33% between 20 - 40 cm (figure 11). This is because nutrients strongly cycled by plants are more concentrated in the topsoil, and therefore it is the soil layer commonly sampled for soil fertility assessments (Jobbágy et al., 2001). Only a few studies assessed soil quality indicators lower than 40 cm (Bole and Gould, 1985; Luo et al., 2004; Matheyarasu et al., 2017; Russell, 1982).

In relation to attributes most frequently assessed within the database (n = 29 papers), most of them were chemical indicators (Figure 12), in which soil pH was the most assessed (68.97%). Within physical indicators, the most assessed was soil bulk density (17.24%). Regarding biological indicators of soil quality, the only two assessed indicators were microbial respiration and microbial diversity, each of them present in 6.90% of the papers. It is important to mention that only indicators present in at least two publications were considered for Figure 12.

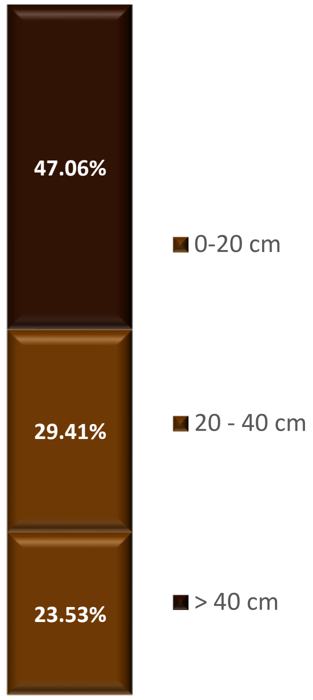

Figure 11. Soil depth most frequently assessed from experimental plots in fields (n = 15).

As reported by Bunemann et al. (2018), chemical indicators of soil quality are usually more frequently assessed in relation to physical and biological indicators. Considering soil quality indicators in soils irrigated with slaughterhouse effluent, the commonness of chemical indicators might be a result of a high nutritional load from effluents, directly influencing soil fertility. Overall, 86.1% of papers assessed chemical indicators, 41.3% physical indicators and 27.5% biological indicators. The work of Seshadri et al. (2014) was the only one that assessed chemical, physical and biological indicators of soil quality (Figure 13). The most frequent combination between components (chemical, physical and biological) of soil quality was chemical and physical (31.3%) as assessed in the works of Shilp et al. (2018), Matheyarasu et al. (2017) and Seshadri et al. (2014). No work assessed soil quality by building or evaluating an analytical soil quality index.

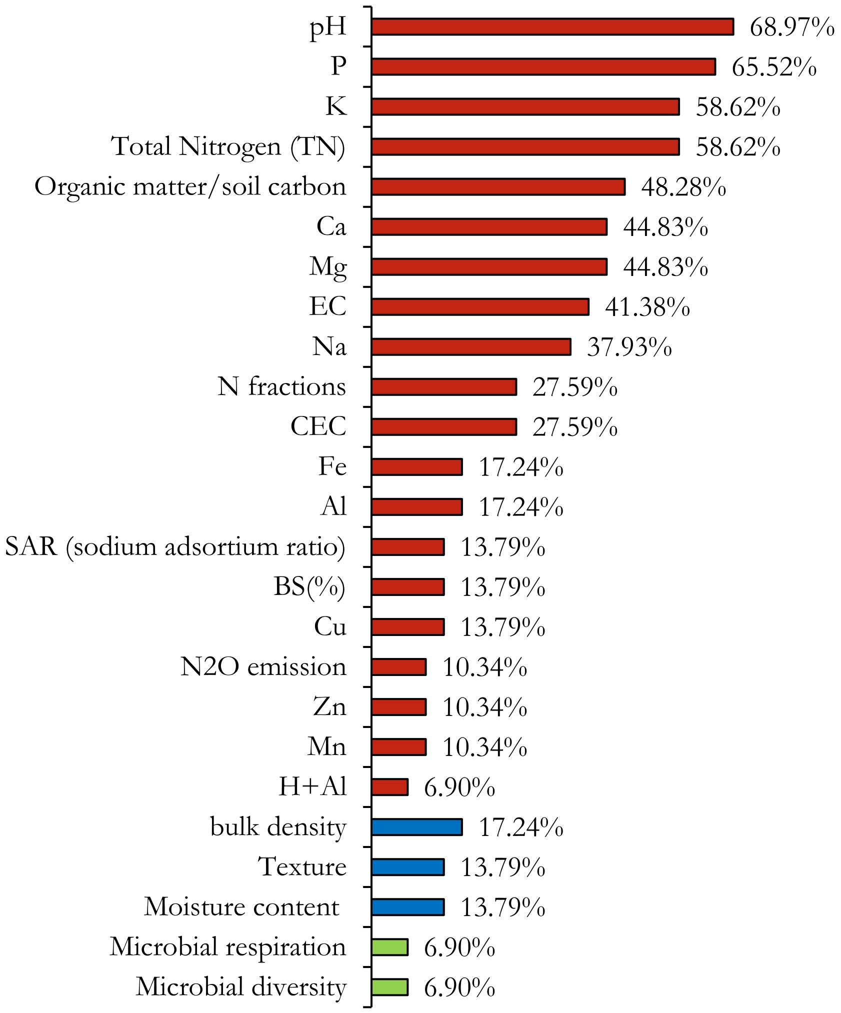

Figure 12. Frequency of appearance of chemical, physical and biological indicators (respectively in red, blue and green) in the publications evaluated in the database. P: phosphorus; K: potassium; Ca: Calcium; Mg: Magnesium; EC: electrical conductivity; Na: sodium; CEC: Cation Exchange Capacity; Fe: Iron; Al: Aluminum; BS: Base saturation; Cu: copper; Zn: Zinc; Mn: manganese; H+Al: potential acidity.

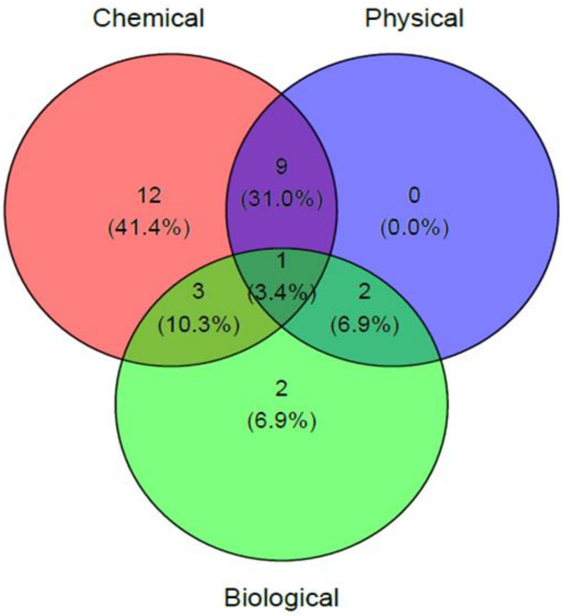

Figure 13. Percentage and total count of studies that assesses chemical, physical and biological indicators of soil quality (n = 29).

2.3.4.1 Impact of irrigation with slaughterhouse effluent on soil quality

According to the frequency of soil quality indicators (Figure 12), the three macronutrients that were most positively-impacted from the application of slaughterhouse effluent were available P/total P (15 studies), available N/total N (10 studies) and available K (8 studies), as shown in the supplementary table 2. This is related to high concentrations of organic matter and these nutrients in slaughterhouse effluents, as previously mentioned (Alabi et al., 2019; Matheyarasu et al., 2016b; N Wells and Whitton, 1970). Regarding the phosphorus, significant increments of its content were reported in layers of 0 - 30 cm (Alabi et al., 2019; Liu and Haynes, 2013), 5 - 35 cm (Matheyarasu et al., 2017) and 0 - 40 cm (Menegassi et al., 2020), as well as when a control treatment was applied, that is, irrigated with tap water (Matheyarasu et al., 2017; Menegassi et al., 2020; Osemwota, 2010; Shilpi et al., 2018).

Similarly, nitrogen concentrations (total N and available N) also increased in the layers of 0 - 10 cm (Liu and Haynes, 2013) and of 0 - 40 cm (Alabi et al., 2019; Matheyarasu et al., 2017; Oliveira et al., 2017) as the application rate of slaughterhouse effluent increased (Oliveira et al., 2017; Shilpi et al., 2018), being higher than control treatments, which were irrigated only with tap water (Osemwota, 2010; Matheyarasu et al., 2016; Matheyarasu et al., 2017; Oliveira et al., 2017). Some works reported an increase in N_2O emissions due to denitrification, which is related to the availability of mineral N and the lability of C in slaughterhouse effluents (Bhandral et al., 2007;

Matheyarasu et al., 2016c; Russell et al., 1993). Similar outcomes can be observed for the increased K concentration from irrigation with slaughterhouse effluents.

Regarding the other exchangeable bases, Ca and Mg, results from the reviewed database differed from one another, although it is important to emphasize that their concentration decrease when Na^+ concentration was increased (Alabi et al., 2019; Liu and Haynes, 2013; Luo et al., 2004). Higher concentrations of Na in slaughterhouse effluents may lead to Na accumulation in the soil, even in the subsoil through leaching (Liu and Haynes, 2013; Luo et al., 2004; Menegassi et al., 2020) and also in Ca^{2+} and Mg2+ displacement (Sumner, 1993). The increase of Na^+ concentration may also lead to clay dispersion, reducing the soil physico-chemical quality and the water availability to plants (Ayers and Westcot, 1985). Such increase in Na concentration was reported by six works within the database (Liu and Haynes, 2013; Luo et al., 2004; Magesan et al., 1999; Menegassi et al., 2020; Oliveira et al., 2017; Wells and Whitton, 1970), followed by increases in the percentage of exchangeable sodium (Liu and Haynes, 2013; Luo et al., 2004) and in sodium adsorption rate (SAR) (Abegunrin et al., 2016). The increment of electrical conductivity was reported by six works in the database (Liu and Haynes, 2013; Matheyarasu et al., 2017; Oliveira et al., 2017; Osemwota, 2010; Balaji Seshadri et al., 2014; Shilpi et al., 2018), which was mainly due to the increase of K^+ and Na^+ concentrations.

In relation to soil pH, nine works reported a decrease of soil pH when the slaughterhouse effluent was applied (Alabi et al., 2019; Da Silva Neto et al., 2013; Guo and Sims, 2003, 2000; Liu and Haynes, 2013; A. V. Luchese et al., 2017; Matheyarasu et al., 2017; Balaji Seshadri et al., 2014) while three others reported an increase in soil pH (Oliveira et al., 2017b; Arku et al., 2014; Osemwota, 2010). Most works relate such decrease in soil pH with oxidation of organic compounds and with the lower pH of the applied effluent (Guo and Sims, 2000; Matheyarasu et al., 2017), as well as the process of nitrification, which releases ions of H^+ (Da Silva Neto, 2013; Guo and Sims, 2003).

Considering other chemical properties as the micronutrients Fe, B and Mn, the works of Matheyarasu et al. (2017), Seshadri et al. (2014) and Osemwota (2010) were the only three that reported positive results after the application of slaughterhouse effluents. Similarly, the works of Alabi et al. (2019), Abegunrin et al. (2016) and Wells and Whitton (1970) were the only three that reported positive impacts for CEC. No significant impact was found for the remaining chemical properties when slaughterhouse effluent was applied.

As previously mentioned, soil physical and biological properties were poorly investigated. The works that reported a positive impact on soil organic matter (SOM) / soil carbon ascribed the results to the large addition of soluble organic matter into the soil from the slaughterhouse effluent

(Wells and Whitton, 1970; Guo and Sims, 2003; Liu and Haynes, 2013). Such increase in SOM may lead to an increase in soil microbial biomass, soil basal respiration, bacterial and fungal diversity and the microbial metabolic quotient (Balks et al., 1997; Liu and Haynes, 2013; Tate, 1973). Other studies reported an increase in the enzyme activity for acid phosphatase (Seshadri et al., 2014) and the increase in the number of earthworms in the soil (Churchman and Tate, 1986).

Although Guo and Sims (2003) attributed the decrease of soil bulk density to the increase of SOM, the work of Alabi et al. (2019) found that the irrigation with slaughterhouse effluent decreased SOM in the subsoil and caused soil compaction in the topsoil due to a high soil moisture. A temporary reduction of soil permeability due to the formation of bacterial biofilm was reported by Balks et al.(1996), although Alabi et al.(2019) reported an increase in saturated soil hydraulic conductivity. Nevertheless, the trend is that the application of treated slaughterhouse effluent influences SOM, which in turn impacts soil bulk density and soil aggregation (Guo and Sims, 2003; Churchman and Tate, 1986).

2.4 Conclusions

Slaughterhouse effluent has been applied through irrigation in crops, and it has a potential to improve soil quality, although its use for irrigation may lead to some issues such as salinization and sodificaton. Within this knowledge area, Australia, New Zealand, Brazil and Nigeria are the countries with the most publications from 1970 to 2022, with the first two having scientific cooperation with each other. The work of Abegunrin et al. (2016) was the most cited within the reviewed database, which was carried out in Nigeria, and the year with the most number of publications was 2017.

In most studies the slaughterhouse effluent was biologically treated and applied in pasture or forage fields. These studies assessed mainly chemical soil properties, from which total/available N, total/available P and exchangeable K the ones that most positively affected soil quality. The work of Seshadri et al. (2014) was the only one that studied chemical, physical and biological soil properties. Soil physical and biological indicators of soil quality were poorly investigated and therefore further research is needed, especially because there have been positive results about biological activity improving soil structure.

In order to expand the use of irrigation with slaughterhouse effluent throughout the world, it is necessary for legislation to indicate critical limits between the effluent characterization parameters, especially when irrigated in agricultural crops intended for human consumption and that also bring the establishment of criteria/parameters to monitor and characterize soils before and after the application of agro-industrial effluents.

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3. SOIL PHYSICAL QUALITY IN SYSTEMS IRRIGATED WITH SLAUGHTERHOUSE EFFLUENT

Abstract

The application of treated slaughterhouse effluent (TSE) can impact soil quality (SQ) due to its characteristics of high concentrations of nutrients and organic matter, but little has been studied about its impacts on soil physics. The objectives of this work were: i) to characterize the irrigation with TSE and its nutrient supply to crops; ii) evaluate the impacts of its irrigation on physical indicators of SQ and iii) evaluate soil physical quality (SPQ) under the imposed experimental conditions. The experiment was arranged in randomized blocks, with five treatments and four replications, namely: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50% and T5 - 25% of the doses of TSE, applied to the black oat/soybean succession between 2020-2022-. In treatment T1 - 0% TSE, the required N doses were supplied via nitrogen fertilizer in the form of urea, by fertigation. For comparison of soil quality indicators, a sixth treatment, T6 – NV, was studied, an area of native vegetation (seasonal semideciduous forest). The evaluated soil physical quality indicators (soil bulk density, resistance to penetration, water filled spore space - WFPS, macroporosity, total porosity, soil structural index, mean weight diameter of soil aggregates - MWD, field-saturated hydraulic conductivity), as well as soil organic carbon, were not impacted by the different doses of TSE. T6 – NV had the best results compared to the other treatments, except for MWD and WFPS. Although these indicators were not directly impacted, treatments T3-75% TSE and T5 - 25% TSE had higher SPQ index compared to treatments T1-0% TSE, T2 - 100% TSE and T4 - 50% TSE, while again T6 - NV was the treatment with the highest score for soil physical quality. Although statistical differences for the physical indicators of soil quality have not been verified separately, the application of TSE to crops can result in the improvement of soil physical quality at adequate doses.

Keywords: Soil physical functions; Abattoir wastewater; Soil health; Meat consumption, Agricultural reuse.

3.1 Introduction

The increased water demand because of an increasing global population may put at risk the amount of water available for agriculture. As population growth increases the need for food, agricultural reuse may be a viable alternative to save fresh water, especially in countries with water scarcity (Qadir et al., 2013; Singh et al., 2009). In addition to providing water, wastewater irrigation is a source of nutrients to crops, which reduces the pressure for synthetic fertilizers (Becerra-Castro et al., 2015; Helmecke et al., 2020).

In this context, meat industry generates large amounts of effluents, 24% of the water consumed for the food and beverage sector (Bustillo-Lecompte and Mehvar, 2015), which needs to be disposed correctly, otherwise may cause water pollution, affecting the surrounding environment and the drinking water consumption. This type of effluent comes from the slaughtering and cleaning processes in slaughterhouses, and its content includes high nutrient concentration (mainly N, P and K), total suspended solids, dissolved salts and possible pathogens (Harris and MacCabe, 2015; Liu and Haynes, 2011; Ribeiro et al., 2013).

The use of slaughterhouse effluent in agriculture is therefore a source of both water and nutrients, which can improve crop yield and enhance soil health (Menegassi et al., 2020; Matheyarasu et al., 2017). In relation to the impact of soil chemical properties on soil quality, works about the application of slaughterhouse effluent on grasslands (Matheyarasu et al., 2017; Menegassi et al., 2020) and croplands (Abengurin et al., 2016), for example, found that it increased the contents of N, P and K in the soil (Liu and Haynes, 2013; Oliveira et al., 2017; Alabi et al., 2019). However, it may also add Na into the soil (Liu and Haynes, 2013; Luo et al., 2004), which can lead to issues such as clay dispersion and decreased soil permeability (Ayers and Westcot, 1989; Almeida-Neto, 2009).

For this reason, it is essential to study the impact of irrigation with slaughterhouse effluent on soil physical quality. The few works about this topic studied impacts on soil permeability (Balks, McLay and Harfoot, 1996), soil water infiltration (Guo and Sims, 2002) and soil bulk density (Alabi et al., 2017; Guo and Sims, 2003). In addition to impacting root growth (Souza et al., 2014), soil physical degradation increases soil erosion and decreases water quality (Issaka and Ashraf, 2017), causing loss of biodiversity (Sylvain and Wall, 2011; Gould et al., 2016; Cherubin et al., 2016).

The hypothesis of this work is that irrigation with treated slaughterhouse effluent (TSE) may impact: i) physical indicators of soil quality; ii) soil physical quality index, in which soils with TSE would be in a condition closer to soils under native vegetation. Thus, this study aimed to: i) characterize the irrigation with TSE and its nutrient supply to crops; ii) assess the impact of TSE irrigation on physical indicators of soil quality; and iii) iii) assess soil physical quality for different doses of TSE.

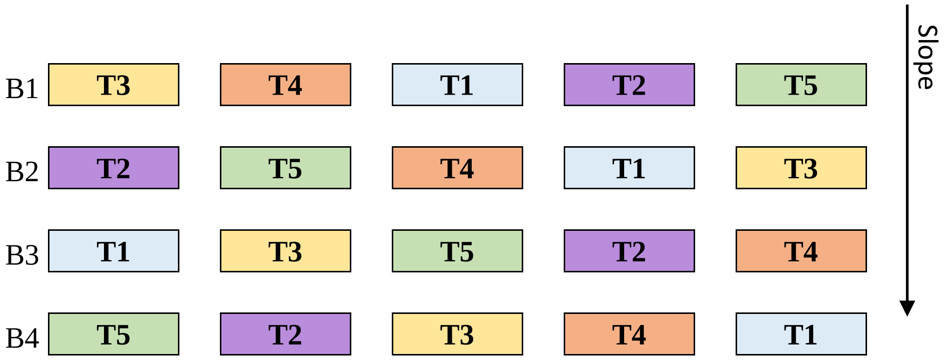
3.2 Material and Methods

3.2.1 Study field and experimental design

The experiment was carried out in the municipality of Pirassununga (21°59’ S, 47°26’ W, 635 m asl), São Paulo State, Brazil, in an area adjacent to a slaughterhouse from the Faculty of Animal Science and Food Engineering at the University of São Paulo, “Fernando Costa” campus. The climate in the region is Cwa, humid subtropical with dry winter and hot summer (Alvares et al., 2013), and the average annual temperature is 20.8°C, with an average annual rainfall of 1298 mm. Prior to carrying out this experiment, Menegassi et al. (2020) evaluated the cultivation of

Coast-cross grass (*Cynodon dactylon* (L.) Pers.) irrigated with treated slaughterhouse effluent (TSE). Information related to soil type, texture, particle density, soil organic carbon, and land use history is shown in Table 1.

The experiment was designed in randomized blocks, with five treatments and four replications, namely: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50% and T5 - 25% of the doses of treated slaughterhouse effluent (TSE) applied by irrigation. In treatment T1 - 0%, the required N were supplied via nitrogen fertilizer in the form of urea, by fertigation. For comparison of soil quality indicators, a sixth treatment T6 – NV, was studied, an area of native vegetation (seasonal semideciduous forest). The study was conducted between 2020 -2022, and a crop rotation of black oat and soybean was established during these two years. Figure 1 shows the experimental design.



B1	**T3**	**T4**	**T1**	**T2**	**T5**
B2	**T2**	**T5**	**T4**	**T1**	**T3**
B3	**T1**	**T3**	**T5**	**T2**	**T4**
B4	**T5**	**T2**	**T3**	**T4**	**T1**

Figure 1. Experimental design where, B: block; T1: irrigation with tap water containing 0% of the N doses required via TSE; T2, T3, T4, and T5 – irrigation with 100%, 75%, 50%, and 25%, respectively, of the doses of N required by the crops via treated slaughterhouse effluent (TSE).

Table 1. Soil classification and soil properties for the experimental area and for the soil under native vegetation.

Soil Classification	**Soil Layer**	**Clay**	**Silt**	**Sand**	**PD**	**SOC**	**Drainage**	**Land use change and management**
	cm		g kg^{-1}		g.cm^{-3}	g kg^{-1}		
Experimental Area								
Eutric Rhodic Ferralsol	0-10	466	144	390	2.86	13.63	well drained	Conversion to pasture (*Brachiaria decumbens*) and cultivated with coastcross grass (*Cynodon dactylon* (L.) Pers.) from 2017 to 2019 by Menegassi et al. (2020)
	10-20	427	162	411	2.88	13.22		
	20-40	482	118	400	2.88	11.29		
Native vegetation								
Eutric Rhodic Ferralsol	0-40	511	126	363	2.83	15.79	well drained	Semideciduous seasonal forest, part of the ecotone Cerrado-Atlantic Forest

[a] Santos (2018). PD – soil particle density; SOC – soil organic carbon

3.2.2 Water source and irrigation characteristics

The effluent used for crop irrigation comes from the Faculty of Animal Science and Food Engineering slaughterhouse at the University of São Paulo, "Fernando Costa" campus. The generated effluent was preliminary treated through a solid separation tank, measuring 3.0 m x 3.20 m and 1.20 m in depth. After this process, the effluent was pumped to the UASB (Upflow Anaerobic Sludge Blanket) reactor for treatment. The UASB reactor implanted in an area adjacent to the cultivation area, had a work volume of 12 m^3, with an application rate ranging from 2 to 4 kg.m^{-3} of chemical oxygen demand (COD) as described by Menegassi et al. (2020). The UASB operated in a continuous flow regime, with 24h of hydraulic retention. After the anaerobic treatment, the effluent was treated by a polishing pond and pumped into a 5000 L reservoir for irrigation.

Sample collection for effluent analysis was carried out weekly according to the National Guide for the Collection and Preservation of Water Samples (CETESB/ANA, 2011) and analyzed according to APHA/AWWA/WEF (2012). The physico-chemical characterization of the effluent was carried out for electrical conductivity, pH, chemical oxygen demand (COD), total solids (TS), nitrogen series (NTK, NH_4+, Norg, NO_3-, NO_2-, NT), phosphorus (P-PO_4-), potassium (K^+), sulfate (SO_4-), calcium (Ca^{+2}), magnesium (Mg^{+2}) and sodium (Na^+). From the concentrations of

Ca, Mg and Na, sodium adsorption ratio (RAS) was calculated by the method described by Ayers & Westcot (1999).

The irrigation system used in the experiment was conventional sprinkler, with sprinklers located at the two diagonal ends of the experimental plots, with an operating angle of 90º, at an initial height of 1 m from the ground. The sprinkler had an adjustable sectoral impact, with a 3.18 mm nozzle, a flow rate of 0.50 $m^3.h^{-1}$ and a working pressure of 25 mH2O. Christiansen's uniformity test was performed, as described by Bernardo et al. (2006), obtaining the respective mean values for each treatment: T1 = 76%; T2 = 77%; T3 = 77%; T4 = 85%; T5 = 80%. These treatments were individualized by solenoid valves, operated by two control panels responsible for irrigation with water and effluent, aiming at the proposed doses for treatments from T1 to T5. The water reservoir had a storage capacity of 3000 L. The centrifugal motor pump set and PVC tubes were responsible for the flow of water to the sprinkler system.

The irrigation management used aimed to maintain soil water tensions between field capacity and critical tensions for crops in the rotation system in the 0 – 20 cm layer of soil. For this purpose, a capacitive sensor of soil moisture was used in the measurements, through frequency domain reflectometry. The irrigation management frequency adopted was 2 days and the humidity in the capacity of field, critical and permanent wilting point obtained by the soil water retention characteristic curve (WRC), at a depth of 0 -20 cm, determined by a pressure chamber (Richards, 1941). The adjustments of the WRC parameters were performed using the model of Van Genuchten (1980), through the RETC software (Van Genuchten et al., 2009).

3.2.3 Crops and fertilization

Information about crop and fertilization was summarized in Table 2. The rotational system included Black oat (*Avena stringosa* Schreb) followed by Soybean *(Glycine max* (L.) Merr.) every year for two consecutive years. It is important to highlight that black oat and soybean residues were removed for the planting operation. Fertilizers applications were fractionated during the crop cycle and provided via fertigation. In addition to the main fertilization describe in Table 2, the following macro-micronutrients were applied during 2020 and 2021: 0.7 kg ha^{-1} of Mn, 2.1 kg ha^{-1} of Zn, 0.046 kg ha^{-1} of B, 1.98 kg ha^{-1} of S, 0.35 kg ha^{-1} of Mg and 3.73 kg ha^{-1} of N.

Table 2: Information about grown crop and main fertilization in the studied area.

Crop	Sowing date (dd/mm/yyyy)	Seeding density	Emergence date (dd/mm/yyyy)	Cultivation period (DAS)	Main Fertilization
			First cycle (2020)		
Black oat (*Avena stringosa* Schreb)	30/07/2020	100 kg ha^{-1}	06/08/2020	60	30 kg ha^{-1} of K_2O for T1-T5, 80 kg ha^{-1} of N from urea in T1
Soybean (*Glycine max* (L.) Merr.)	15/10/2020	15 seeds m^{-1}	19/10/2020	120	20 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O in T1-T5
			Second cycle (2021)		
Black oat (*Avena stringosa* Schreb)	21/05/2021	100 kg ha^{-1}	28/05/2021	110	60 kg ha^{-1} of K_2O and 40 kg ha^{-1} of P_2O_5 from T1-T5, 60 kg ha^{-1} of N from urea in T1
Soybean (*Glycine max* (L.) Merr.)	29/10/2021	15 seeds m^{-1}	07/11/2021	120	20 kg ha^{-1} of P_2O_5 and 30 kg ha^{-1} of K_2O in T1-T5

T1 = 0% treated slaughterhouse effluent (TSE), T2 = 100% TSE, T3 = 75% TSE, T4 = 50% TSE and T5 = 25% TSE

3.2.4 Soil physical attributes evaluation

Soil sampling and field assessments were carried out in July 2020 (characterization) and in March 2022. In each one of the four replicates from systems T1 to T5, one sampling point was chosen in the center of each plot, in the interrow, avoiding anthills or compaction zones. In the native vegetation, the soil was sampled in triplicates, in three fragments of native vegetation, set 5 m apart from each other, avoiding sampling next to anthills, animal burrows and tall trees, as described by Cherubin et al. (2016). For each sampling point, a small trench of 30 x 30 x 40 cm was dug, and samples were taken in the layers of 0-10, 10-20 and 20-40 cm, totaling 23 sampling points and 69 undisturbed soil samples. The maximum soil depth of 40 cm was used due to the effective rooting depth from crops in rotational systems (Fan et al., 2016; Myers, 1980).

The undisturbed soil samples from soil cores were used to determine a range of physical indicators of soil quality. The cores were saturated with water and soil microporosity was then determined as the water content at -6 KPa. Soil bulk density (BD, $g.cm^{-3}$) was determined as fraction between the weight of the soil dried for 48 h at 105 °C and the core volume, which was about 97 cm^3 (Grossman and Reinsch, 2002). Soil particle density (PD, $g.cm^{-3}$) was assessed using a helium pycnometer from 5g of disturbed soil samples, as described by Flint and Flint (2002). From soil bulk density and particle density values, it was possible to calculate total porosity (TP, $m^3.m^{-3}$) as TP = 1 – (BD/PD). Soil macroporosity (Map, $m^3.m^{-3}$), was determined by subtracting the saturated water content by field capacity (-6 KPa). Water-filled pore space (WFPS) was assessed as the relation between the volumetric water content at field capacity (-6 KPa) and total porosity, as described by Wienhold et al. (2009). As suggested by Reynolds et al. (2009), the soil structural index (SSI, %) was determined using the following pedotransfer function: SSI = [(SOC x 1.724) / (silt + clay)]*100, where SOC is the soil organic carbon content (SOC, g kg-1), 1.724 is a conversion factor from SOC to soil organic matter (SOM), and silt and clay contents are in g kg^{-1}.

Soil resistance to penetration (RP, MPa) measurements were carried out in five replicates in each experimental plot and in the three fragments of native vegetation by using a digital penetrometer (Penetrolog). Such measurements were performed as close as possible to field capacity, in which the gravimetric water content was constantly monitored. Saturated soil hydraulic conductivity (Kfs, $mm.h^{-1}$) was measured on-site using the method BEST - Beerkan Estimation of Soil Transfer, as described by Lassabatère et al. (2006). In order to do so, a steel cylinder (7 cm height, 16 cm diameter) was placed 1 cm down into the soil and 150 mL of water was added in each run for eight times, or up to the number of times needed for the infiltration rate to reach a steady state. Saturated soil hydraulic conductivity was thereafter estimated by using the algorithm proposed by Bagarello et al. (2014). This procedure was performed in duplicates in the experimental plots, and the average value was used in this work.

Mean-weighted diameter of water-stable aggregates (MWD, mm) was determined according to van Bavel (1950). Undisturbed soil samples of 10 cm^3 were sieved through a 9520 μm sieve and saturated for 24h. The aggregates were thereafter placed at the top of a set of three sieves, 2000, 250 and 53 μm in an apparatus for vertical oscillation in 42 rpm by 15 min (Yoder, 1936). Mean weight diameter was calculated as the weighted sum of the occurrence of each class of aggregate size.

The initial characterization of the physical indicators of soil physical quality for both the experimental area and the native vegetation is shown in Table 3.

Table 3. Initial characterization (June 2020) of physical indicators of soil quality and soil organic carbon in the experimental field and in the native vegetation.

Location	Depth cm	BD $g.cm^{-3}$	RP MPa	WFPS $m^3.m^{-3}$	Map $m^3.m^{-3}$	TP $m^3.m^{-3}$	SSI %	MWD mm	SOC $g\ kg^{-1}$
Experimental field	0-10	1.19	1.17	0.595	0.239	0.587	3.92	4.41	13.64
Native vegetation	0-10	1.01	0.98	0.540	0.298	0.648	4.93	4.39	20.83
Experimental field	10-20	1.32	1.65	0.726	0.150	0.540	3.80	4.21	13.22
Native vegetation	10-20	1.09	1.80	0.580	0.261	0.617	4.76	3.92	19.79
Experimental field	20-40	1.31	1.72	0.709	0.160	0.545	3.25	3.75	11.29
Native vegetation	20-40	1.03	1.73	0.573	0.274	0.640	5.47	3.95	15.41

BD: Bulk density; RP: soil resistance to penetration; Kfs: Field-saturated hydraulic conductivity; WFPS: water filled pore space; Map: Macroporosity; TP: total porosity; MWD: mean weight diameter of soil aggregates; SSI: Stability structural index; SOC: soil organic carbon.

3.2.5 Soil physical quality index calculation

A soil physical quality index (SPQI) was calculated based on the above-mentioned physical indicators of soil quality combined in four soil functions: i) support root growth; ii) supply water for plants, iii) soil aeration, iv) ability to resist to soil degradation, as previously used by Cherubin et al. (2016). For each of the presented functions, the following indicators were considered: F(i) - BD, RP, F(ii) - Kfield, WFPS, F(iii) - Map, TP and F(iv) - MWD, SSI. The mean values for each indicator were calculated for the 0-40 cm soil layer, followed by scoring them into unitless values ranging from 0 to 1 based on linear transformations suggested by Andrews et al. (2002), values of 1 being the best-case scenario. For indicators classified as "more is better", each observation was divided by the highest value within the dataset; for "less is better", the lowest value within the dataset was divided for each observation and for "mid-point optimum", an ideal value was established, observations were scored either as "more is better" for values lower than the ideal value, and as "less is better" for values higher than the ideal value.

After scoring, each indicator was assigned and weight (0.5) and summed within each one of the four soil functions listed above. Each function was thereafter multiplied by its weight (0.25) and summed to compose the final soil physical quality index for the 0-40 cm layer (Supplementary Table 1 – Appendix B).

3.2.6 Data analyses

The dataset was initially tested for normality by the Shapiro-Wilk test ($p<0.05$) and whenever necessary data was transformed according to Box and Cox (1964). Analysis of variance (Anova) was performed ($p<0.05$) and whenever results were significant, they were compared by using regression analysis (treatments T2 to T5) and by analysis of contrast with the Scheffé test ($p<0.05$).

Scores for physical indicators of soil quality and the soil physical quality indexes in each treatment were submitted to the Scott-Knott test ($p<0.05$). The Pearson correlation coefficient was calculated between soil physical indicators and SOC to analyze the relationship between variables and a Principal Component Analysis (PCA) was also performed (Hotelling, 1933). The statistical analyses were performed using the environment RStudio 1.4.1103 and the software Sisvar 5.6 (Ferreira, 2019).

3.3 Results

3.3.1 Slaughterhouse effluent, irrigation and nutrient supply

The physico-chemical characterization of the slaughterhouse effluent applied during the experiment is shown in Table 3. According from the resolution number 420 from the Brazilian National Environment Council (Conama, 2011), the effluent would not be suitable for disposal in water bodies as the ammoniacal nitrogen content is over the critical limit (20 $mg.L^{-1}$). However, according to the resolution number 503 (Conama, 2021) regarding the reuse of agro-industrial effluents, the effluent is classified as suitable for agricultural reuse because as the resolution mentions that parameters of agronomic interest are not required to follow the resolution 420 from 2011.

According to Ayers and Westcot (1989) there is a moderate restriction about the impacts of applying this type of effluent in relation to soil water infiltration whenever sodium adsorption ratio is higher than 3 $(mmol.L^{-1})^{-1/2}$ and electrical conductivity 0.7 $dS.m^{-1}$. In this study, however, there is no risk for salinization or sodification was 0.55 $dS.m^{-1}$ and SAR 2.11 $(mmol.L^{-1})^{-1/2.}$

Treatment T2 – 100% TSE was the one with highest irrigation depth and Ntot supply for both crops and cycles (Table 5), followed by treatments T3 – 75%, T4 – 50 % and T5 – 25%, as expected. Total irrigation depth for the period from 2020 to 2022 for two cycles of black oat and two of soybean is shown in Figure 2. Nutrient supply is shown in Table 4. It is important to note that there was no nitrogen fertilization in the soybean for the treatment 0% TSE. The N supply came from the micronutrient application.

Table 4. Mean values followed by standard deviation for the physico-chemical characterization of the treated slaughterhouse effluent (TSE) and tap water (TW) between 2020 and 2022.

Parameter		**TSE**			**SW**
N-NH$_4^+$	(mg.L^{-1})	45.81	±	20.18	0.00
N-NTK	(mg.L^{-1})	60.53	±	31.60	1.68
N-NO$_3^-$	(mg.L^{-1})	1.13	±	0.71	0.80
N-NO$_2^-$	(mg.L^{-1})	0.13	±	0.14	ND
N-TN	(mg.L^{-1})	61.21	±	31.15	2.48
Ca^{+2}	(mg.L^{-1})	18.19	±	3.82	6.85
Fe^{+2}	(mg.L^{-1})	1.42	±	2.01	ND
Mg^{+2}	(mg.L^{-1})	1.83	±	0.50	0.57
Mn^{+2}	(mg.L^{-1})	0.09	±	0.04	ND
S-SO$_4$	(mg.L^{-1})	2.55	±	1.86	ND
Na$^+$	(mg.L^{-1})	34.11	±	9.58	0.80
K$^+$	(mg.L^{-1})	14.32	±	3.38	0.80
P-PO$_4^-$	(mg.L^{-1})	5.75	±	2.90	0.08
pH	-	7.87	±	0.66	6.39
EC	(dS.m^{-1})	0.55	±	0.17	0.09
SAR	(mmol.L^{-1})$^{-1/2}$	2.11	±	0.68	0.26
COD	(mg.L^{-1})	461.12	±	238.76	-
TS	(mg.L^{-1})	542.08	±	303.08	159.37
TDS	(mg.L^{-1})	218	±	30.47	-

TKN: total Kjeldahl nitrogen; TN: total nitrogen; EC = electrical conductivity; SAR: sodium adsorption ratio; COD: chemical oxygen demand; TS: total solids; TDS: total dissolved solids; ND: not detectable.

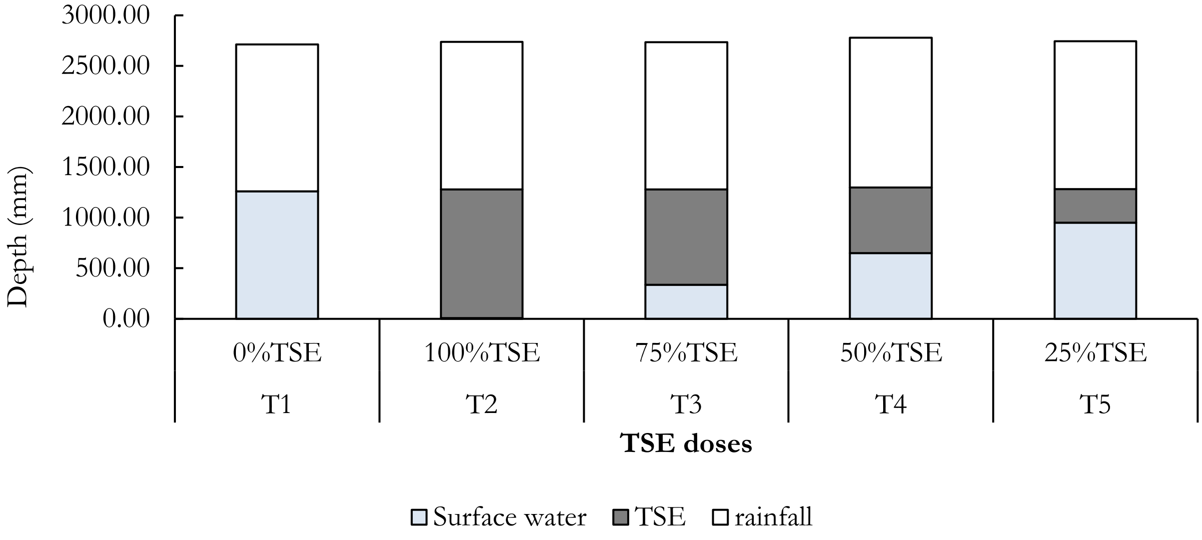


Figure 2. Depth for tap water (TW), treated slaughterhouse effluent (TSE) and rainfall, between 2020-2022.

According to Raij et al. (1996), 20 kg ha^{-1} of N is recommended for black oat during sowing and after each cut. In the first cycle of black oat (2020) 80 kg ha^{-1} by means of urea was applied in T1 – 0% TSE, while 60 kg ha^{-1} was applied in the second cycle. It is possible to verify that during the first crop cycle for treatments T2 to T5, nutrient supply was higher than the recommended for fertilization (Table 5). Similarly, nutrient supply in treatments T2 to T4 were higher than the recommended in the second cycle (2021), while it was lower for T5 (52.58 kg ha^{-1}). In relation to soybean, Cordeiro and Echer (2019) suggest a supply of 50 kg ha^{-1} of N combined with biological nitrogen fixation to increase soil N and crop yield. In this view, treatment T5 is within the recommended range.

Table 5. Supply of macro and micronutrients via treated slaughterhouse effluent (TSE) and conventional fertilization.

Nutrients	TN	Ca	Fe	Mg	Mn	S	Na	K	P	B	Zn
	Kg ha^{-1}										
Treatments	Black oat (2020)										
T1	87.34	28.44	0.00	2.38	0.00	0.00	3.32	33.32	0.33	0.00	0.00
T2	411.78	78.82	3.75	6.53	0.25	8.02	154.82	90.57	5.96	0.00	0.00
T3	298.71	56.60	2.70	5.35	0.18	5.76	112.11	89.38	4.37	0.00	0.00
T4	212.39	39.63	1.89	4.48	0.12	4.03	79.51	88.93	3.16	0.00	0.00
T5	123.31	22.14	1.05	3.54	0.07	2.25	45.88	87.72	1.91	0.00	0.00
	Black oat (2021)										
T1	68.90	23.84	0.00	1.99	0.00	0.00	2.78	62.78	40.28	0.00	0.00
T2	188.15	75.09	2.85	7.62	0.42	14.03	97.36	108.78	59.31	0.00	0.00
T3	144.43	62.82	2.15	6.27	0.32	10.60	74.27	97.57	54.66	0.00	0.00
T4	91.52	45.94	1.32	4.47	0.20	6.52	46.51	83.95	49.10	0.00	0.00
T5	52.58	35.99	0.70	3.34	0.10	3.43	25.86	73.98	44.93	0.00	0.00
	Soybean (2020/2021)										
T1	11.17	19.93	0.00	2.02	0.70	1.98	2.33	62.33	20.23	0.05	2.10
T2	120.64	53.59	7.78	5.07	0.87	8.31	106.46	104.37	41.36	0.05	2.10
T3	93.32	45.30	5.83	4.32	0.83	6.73	80.44	93.88	36.08	0.05	2.10
T4	71.31	40.17	4.22	3.84	0.79	5.42	59.06	85.37	31.72	0.05	2.10
T5	39.53	29.42	1.99	2.87	0.74	3.60	29.10	73.20	25.65	0.05	2.10
	Soybean (2021/2022)										
T1	9.02	14.17	0.00	1.54	0.70	1.98	1.65	31.65	20.17	0.05	2.10
T2	168.18	37.38	3.14	4.06	0.88	6.96	71.57	60.09	37.77	0.05	2.10
T3	128.43	31.67	2.36	3.44	0.83	5.72	54.10	53.00	33.37	0.05	2.10
T4	96.21	28.27	1.71	3.03	0.80	4.69	39.88	47.31	29.77	0.05	2.10
T5	50.06	20.76	0.81	2.24	0.75	3.26	19.65	39.02	24.69	0.05	2.10
	Total (2020 - 2022)										
T1	176.44	86.38	0.00	7.93	1.40	3.96	10.09	190.09	81.01	0.09	4.20
T2	888.75	244.88	17.52	23.28	2.42	37.32	430.20	363.83	144.40	0.09	4.20
T3	664.89	196.40	13.04	19.38	2.16	28.81	320.92	333.82	128.48	0.09	4.20
T4	471.44	154.00	9.14	15.82	1.91	20.66	224.97	305.56	113.75	0.09	4.20
T5	265.48	108.31	4.55	11.99	1.66	12.54	120.48	273.93	97.18	0.09	4.20

TN = total nitrogen, T1 = 0%TSE, T2 =100%TSE, T3 = 75%TSE, T4 = 50%TSE and T5 = 25%TSE.

3.3.2 Physical indicators of soil quality and SOC

For all physical indicators of soil quality assessed (BD, RP, WFPS, Map, TP, SSI, MWD and Kfs), no statistical difference was found through regression analysis between treatments 25, 50, 75 and 100% of TSE (Figure 3). Statistical differences were found in the analysis of contrast between these treatments and the treatments T1 – 0%TSE and T6 – NV, except for WFPS and MWD (Table 6). No significant interactions were found between treatments and studied layers (0-10, 10-20 and 20-40 cm) for any indicator. However, highest values of BD and PR were found in the 10 – 20 cm layer, while highest values for TP, Map, MWD and SSI were found in the 0 – 10 cm layer. No differences for soil depths were found for WFPS.

Table 6. Mean values for physical indicators of soil quality and SOC for treatments T1 – 0% TSE, T2 – 100% TSE, T3-75% TSE, T4 – 50% TSE, T5 – 25% TSE and T6 – NV.

Treatments	BD		RP		WFPS	Map		TP		MWD		SSI		SOC		GWC
	g cm^{-3}		MPa		-	$m^3\,m^{-3}$		$m^3\,m^{-3}$		mm		%		g kg^{-1}		g g^{-1}
T1	1.290	#	0.817	#	0.505	0.133	#	0.551	#	4.058		3.444	#	11.974	#	0.252
T2	1.341	#	0.799	#	0.532	0.113	#	0.534	#	3.686		3.447	#	11.983	#	0.256
T3	1.295	#	0.777	#	0.492	0.144	#	0.549	#	3.873		3.622	#	12.338	#	0.255
T4	1.301	#	0.791	#	0.529	0.112	#	0.547	#	3.920		3.474	#	12.079	#	0.260
T5	1.318	#	0.771	#	0.512	0.151	#	0.541	#	4.140		3.539	#	12.303	#	0.255
T6	1.042		1.502		0.564	0.278		0.635		4.084		5.055		18.677		0.242
CV (%)	6.34		9.99		12.04	27.21		5.09		14.62		8.36		6.35		6.13
0-10	1.225	b	0.660	c	0.487	0.173	a	0.573	a	4.454	a	3.967	a	14.136	a	0.255
10-20	1.308	a	1.070	a	0.535	0.135	b	0.544	b	4.002	ab	3.667	b	13.170	b	0.251
20-40	1.289	ab	0.910	b	0.540	0.142	b	0.551	ab	3.408	b	3.488	b	11.659	c	0.252
CV (%)	7.84		6.19		16.80	31.46		6.27		18.16		6.84		8.32		6.13

BD: Bulk density; RP: soil resistance to penetration; WFPS: water-filled pore space; Map: Macroporosity; TP: total porosity; MWD: mean weight diameter of soil aggregates; SSI: Stability structural index; SOC: soil organic carbon; GWC: Gravimetric Content of Water; CV: coefficient of variation. Means followed by the hashtag differ from treatment T6 (Scheffé test, $p<0.05$). Means followed by different letters in columns, shows statistical differences by Scott-Knott test ($p<0,05$).

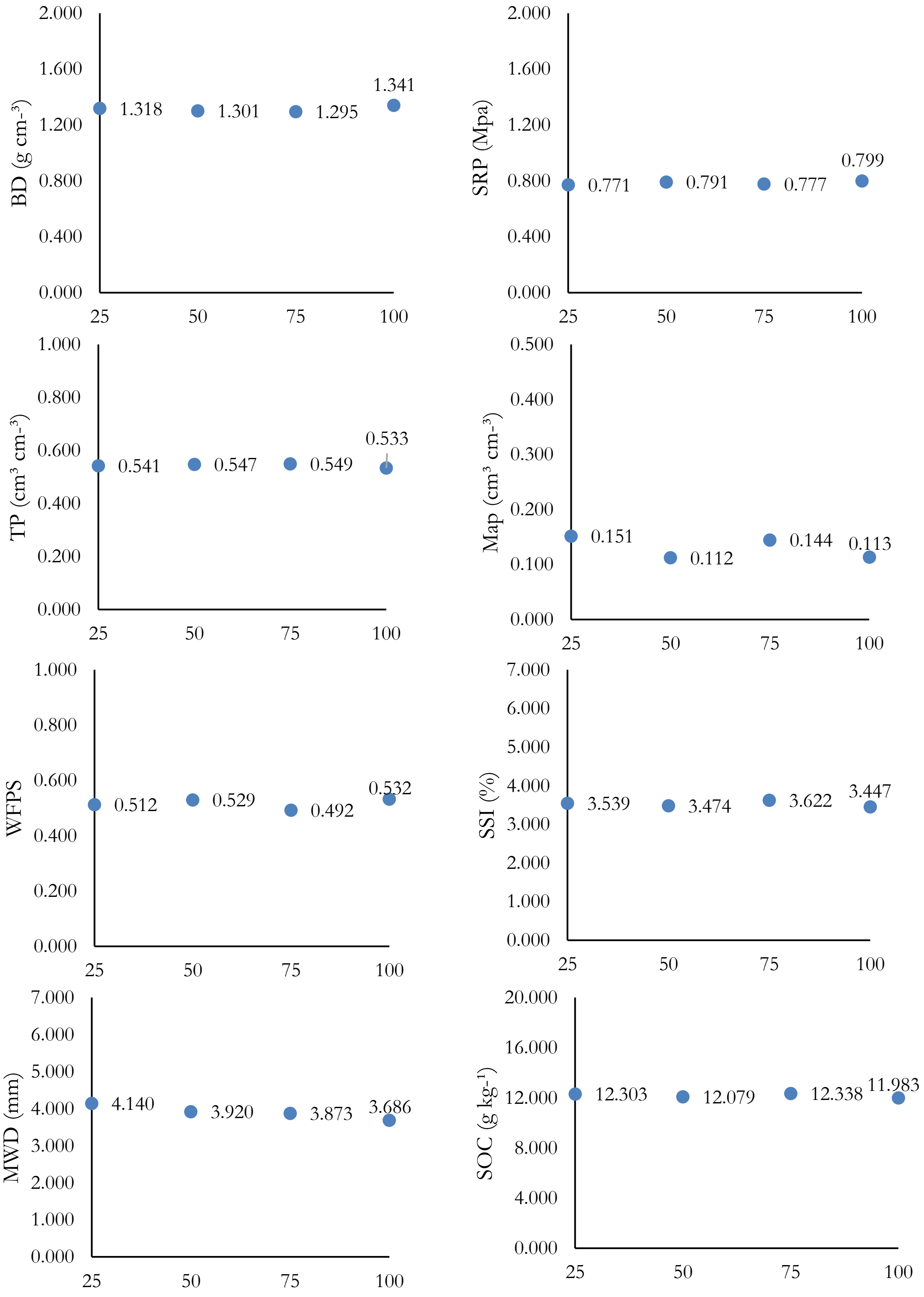


Figure 3. Physical indicators of soil quality for treatments T2 – 100% TSE, T3- 75% TSE, T4 – 50% TSE and T5-25% TSE. There were no regression adjustments for these treatments. BD: Bulk density; RP: soil resistance to penetration; WFPS: water-filled pore space; Map: Macroporosity; TP: total porosity; MWD: mean weight diameter of soil aggregates; SSI: Stability structural index; SOC: soil organic carbon.

Similarly, treatments 0, 25, 50, 75 and 100% of TSE differed from T6 – NV for SOC, but there was no interaction between treatments and soil layers (Table 5). As expected, the 0 – 10 cm layer was the one with higher means. In relation to the field-saturated soil hydraulic conductivity (Figure 4), no significant differences were found between doses of TSE. However, there was a difference between these treatments and T6 – NV, according to Scheffé test ($p<0.05$).

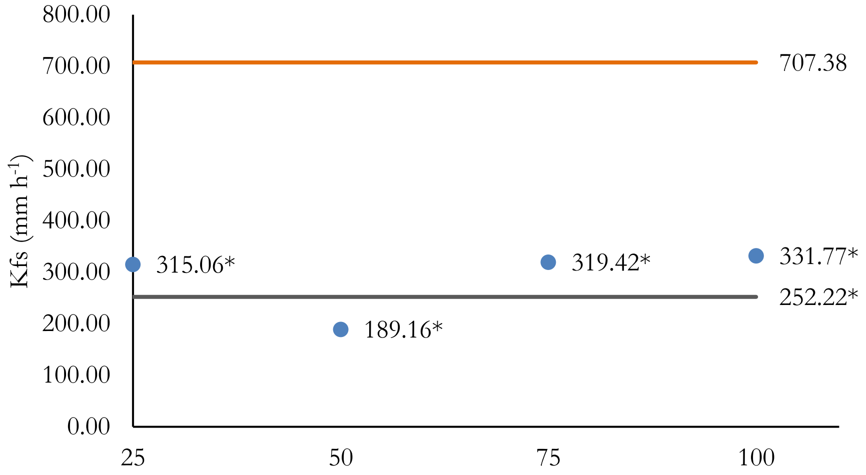


Figure 4. Field saturated hydraulic conductivity (Kfs) for treatments T1 – 0%TSE (grey line), T2 – 100%TSE, T3 – 75%TSE, T4 – 50%TSE, T5 – 25%TSE and T6 – NV (orange line). Means followed by asterisks statistically differ from T6 – NV according to the Scheffé test ($p<0.05$).

3.3.3 Correlation between physical indicators of soil quality and SOC

As shown in Figure 5, the highest significant ($p<0.05$) correlation was found between TP and BD (negatively correlated), which was expected because TP was calculated from BD values. Furthermore, soil macroporosity was also strongly negatively correlated with BD, which is a result of soil compaction reducing the pore network (Batey, 2009; Hamza and Anderson, 2005). BD was only negatively correlated with other soil quality indicators, with strong correlations between all expect WFPS and MWD. Apart from BD, all others were only positively correlated with each other (Figure 5).

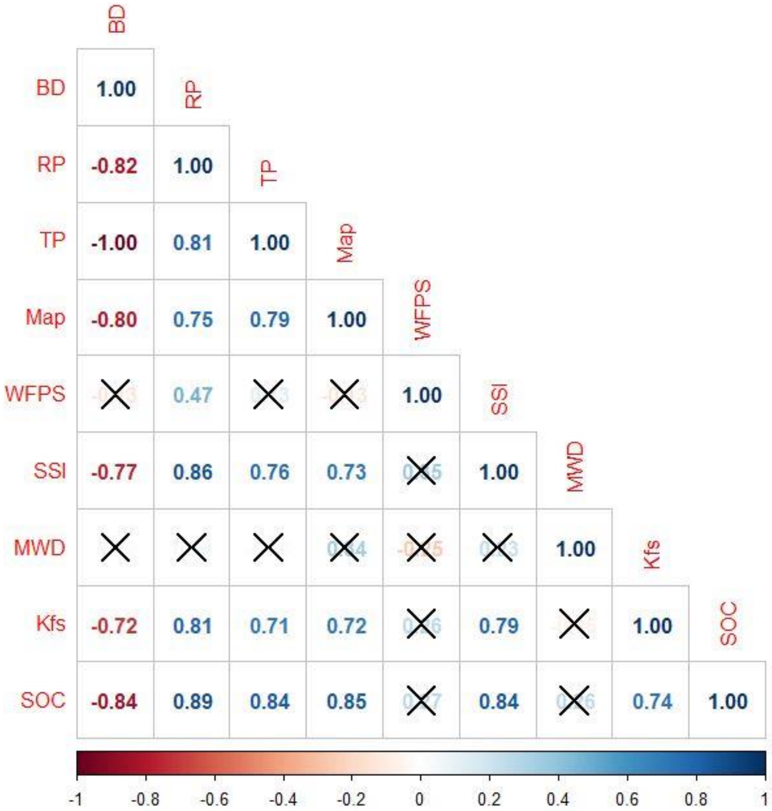


Figure 5. Pearson correlation matrix between physical indicators of soil quality and SOC. Values crossed with an "X" are non-significant ($p<0.05$). BD: Bulk density; RP: soil resistance to penetration; WFPS: water-filled pore space; Map: Macroporosity; TP: total porosity; MWD: mean weight diameter of soil aggregates; SSI: Stability structural index; SOC: soil organic carbon; Ksf: field-saturated hydraulic conductivity.

According to the principal component analysis (PCA), the first component – PC1, with eigenvalue of 5.89, account for 65.5% of data variability. PC2, with eigenvalue of 1.46, accounted for 16.2% of data variability. PC1 was mostly influenced by BD, SOC, RP and Map, while PC2 was mostly influenced by MWD and WFPS (Figure 6). As also shown in the correlation matrix, it is possible to note that BD was negatively correlated with both TP and SSI. By analyzing PC1, T6 – NV was the treatment with highest PCA scores, separated from all other treatments. By analyzing PC2, it is important to note that there was a clear distinction between T1 – 0%T SE and T2 – 100%TSE.

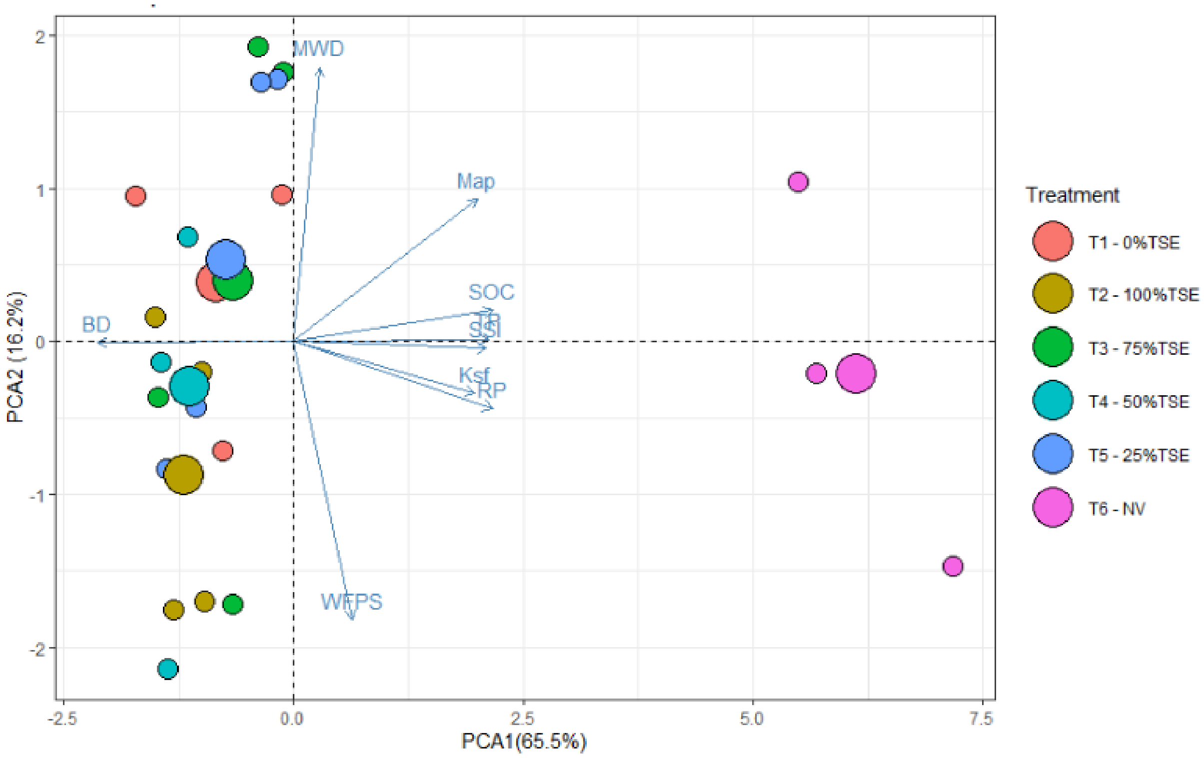


Figure 6. Biplot graph representing the principal component analysis (PCA) for each indicator is characterized by blue arrows and for each studied treatment (T1 – 0% TSE, T2 – 100% TSE, T3 – 75% TSE, T4 – 50% TSE, T5 – 25% TSE and T6 – NV) in colored circles.. BD: Bulk density; RP: soil resistance to penetration; WFPS: water-filled pore space; Map: Macroporosity; TP: total porosity; MWD: mean weight diameter of soil aggregates; SSI: Stability structural index; SOC: soil organic carbon; Ksf: field-saturated hydraulic conductivity.

3.3.4 Soil physical quality

Similar to what was plotted by PCA, the treatment T6 – NV was the one that most differed from other treatments, accounting for the highest SPQI. Soil physical quality was higher in both T3 and T5 than in T1, T2 and T4, in which no different was found between these three. Such results suggest that between applied effluent doses, the ones more beneficial for physical soil quality were 25%TSE and 75%TSE (Figure 7).

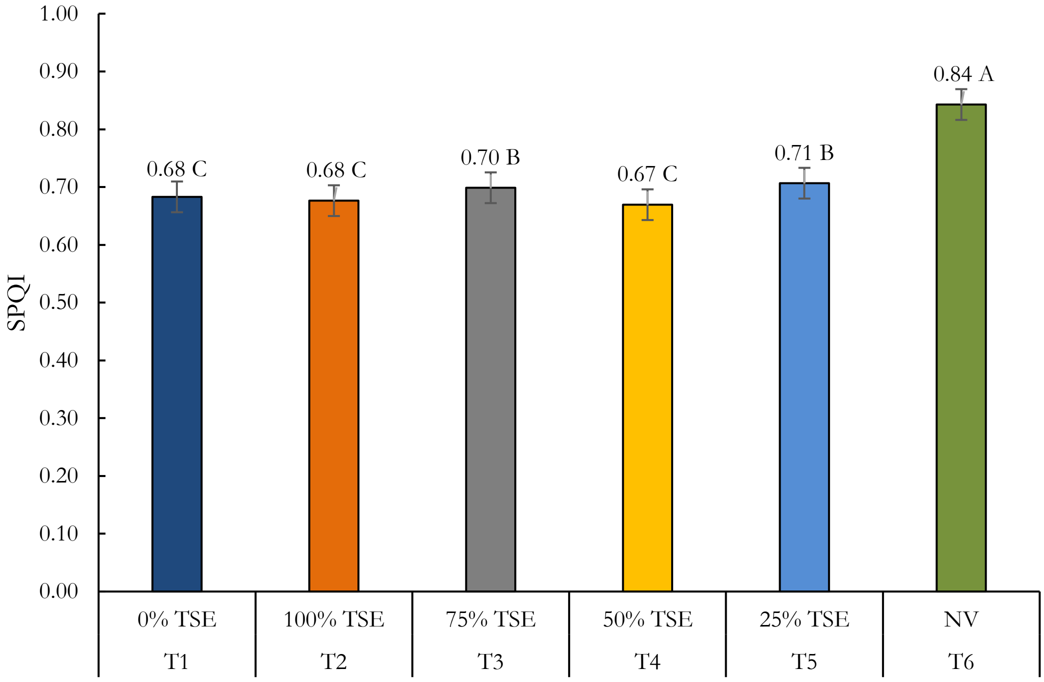


Figure 7. Soil Physical Quality Index (SPQI) after a two-year crop rotation of black oats and soybeans (T1 – T5), compared to T6 (NV – Native vegetation). Means followed by the same letter do not differ according to Scott-Knott test ($p<0.05$).

By breaking down the soil quality index into each one of the four soil functions, f(i): support root growth, f(ii): supply water for plants, f(iii): soil aeration, f(iv): ability to resist to soil degradation, and further analyzing the scores in each function, it is noticeable that T6 – NV only scored lower for f(i), root growth (Figure 8). This may be related to the high RP values, as a result of the lower absolute values of GWC (gravimetric water content), as shown in table 6. As reported by Tormena et al. (2022) and Silveira et al. (2010), RP integrates the effects of soil density and moisture, and the lower the water content in the soil, the more cohesive its particles will be. Regarding the other three soil functions, T6 scored significantly higher than all other treatments. In relation to f(iii), soil aeration, treatments T1, T3, and T5 scored better than T2 and T5.

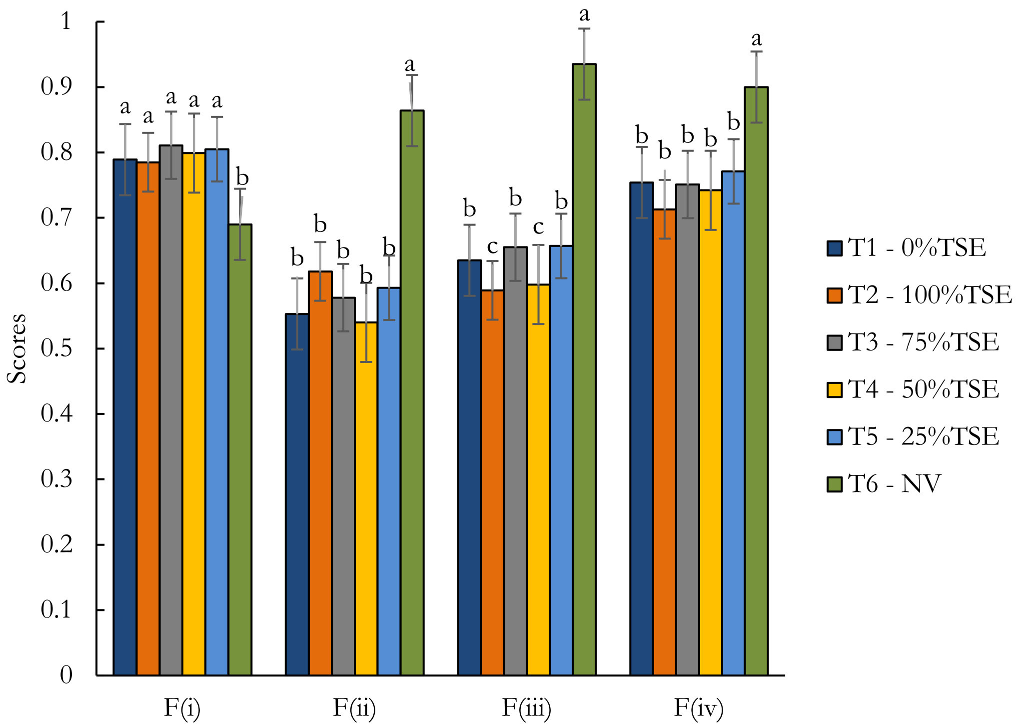


Figure 8. Mean scores for soil physical functions F(i): support root growth, F(ii): supply water for plants, F(iii): soil aeration, F(iv): ability to resist to soil degradation, which were used to compose the SPQI – soil physical quality index. Mean values followed by the same letter do not differ by the Scott-Knott test ($p<0.05$). The standard deviation is shown in the error bars.

3.4 Discussion

3.4.1 Physical indicators of soil quality

The lack of differences for physical indicators of soil quality between doses of TSE (25, 50, 75 and 100%) in comparison to T1 – 0% TSE highlights the potential to replace synthetic fertilization for TSE, as no deleterious effect was observed when crops were irrigated with slaughterhouse effluent. Treatment T6 – NV had the best results for physical indicators of soil quality (except RP, WFPS and MWD) very likely as a result of its higher SOC content (Table 3, Table 5).

Similar to results found in this work, Churchman and Tate (1986) and Bhandral et al. (2007), did not find differences for MWD and WFPS, respectively, from TSE application. By studying soil aggregation, porosity and soil water infiltration for four years with different doses of treated sewage effluent (0%, 11%, 31%, 60%, 87%, and 100%), Coelho et al. (2020) did not find differences in these soil physical indicators. Almeida et al. (2018), assessed the application of swine wastewater for one year and they also did not find changes in soil physics. Such outcome suggests that changes in soil physics in relation to effluent application may require longer periods of time.

Although doses of TSE did not change the soil physical condition in this study, other works mention differences in soil physical quality for long-term application of TSE. This includes changes in soil permeability (Balks, McLay and Harfoot, 1996), decrease in soil water infiltration (Guo and Sims, 2002) and changes in soil bulk density, either to increase (Alabi et al., 2017) or decrease it (Guo and Sims, 2002). Such changes are the result of stimulating soil microbial activity from adding TSE, which leads to the formation of a biofilm over the soil surface (Balks, McLay and Harfoot, 1996), as well as the increment of sodium due to the high sodium content in the effluent, impacting soil structure (Guo and Sims, 2002). Regarding soil bulk density, its decrease (Guo and Sims, 2002) is related to addition of SOC and the increased soil microbiota and macrofauna activity, while its increase (Alabi et al., 2017) is related to the higher soil moisture from TSE application, which may lead to soil disruption and compaction.

It is important to note that although no statistical time comparison was carried out from physical indicators of soil quality, contrasting the results from Table 2 and Table 5, it is possible to note a decrease in RP, WFPS and Map between 2020 and 2022. The decrease in soil macroporosity may be related to the increment of dissolved salts, which causes pore clogging (Bedbabis et al., 2014; Alves et al., 2015). The decrease in RP may be related to the increment and maintenance of soil moisture, because in the initial characterization the field had not received any irrigation.

An important point about SSI is that is that the results, both for the initial characterization as well as after TSE application, was lower than 5% in all treatments, T6 – NV included. Such outcome may suggest a soil susceptibility for physical degradation, as mentioned by Cherubin et al. (2016). Thus, it is very important to monitor SAR and EC to avoid events that cause soil disruption, such as clay dispersion.

3.4.2 Correlation between soil quality indicators

Strong correlations between physical indicators of soil quality were also reported by Cherubin et al. (2016) and Valani et al. (2022). In this work, no correlation was found for either WFS or MWD, and the lack of correlation for MWD was also reported by Valani et al. (2022). Only a few works studied the impact of TSE irrigation on soil physical quality. The work of Churchman and Tate (1986) reported that TSE application does not increase MWD, but it helps to maintain adequate values due to increased addition of organic matter.

In this view, SOC is of primary importance for the maintenance of soil physical quality as it strongly influences the formation and stabilization of soil aggregates (Gumus and Seker, 2015; Lavelle et al. 2020). Lavelle et al. (2020) suggests a cyclical effect between soil aggregation and the maintenance of soil organic matter content. The authors mention that by influencing soil aggregation, adequate levels of SOM/SOC build up ideal conditions for organo-mineral complexation for SOM within soil aggregates. This leads to SOM protection against degradation by microorganisms, and therefore result in more humified SOC forms, which are essential for the maintenance of SOM contents.

Although previous works mentioned an increase in SOM in the topsoil (0 -20 cm) from TSE irrigation (Guo and Sims, 2003; Matheyarasu et al., 2017; Liu and Haynes, 2013), the increase in TSE doses in this work did not lead to increments of SOC content, which may be related to the duration of the experiment. The work of Smith, 2004 mentions that the number of years necessary to detect changes in SOC varies with field conditions and the SOC assessment method but suggests that most changes are noticeable from 6 to 10 years after experiments where SOC content is expected to change are set.

3.4.3 Soil physical quality

The highest soil physical quality index was found for the 0 – 40 cm layer was found for treatment T6 – NV, where it performed 84% of its functioning capacity. It is possible that maximum capacity was not reached in this native vegetation due to its high means for RP. This also impaired its score for f(i), support root growth, in relation to all other treatments (0.69). However, for all other functions, the scores were close to or higher than 0.9, which suggest that the high soil organic matter content in this treatment improved soil structure and aggregation, leading to a higher soil water infiltration, soil aeration and resistance to degradation.

Although different doses of TSE did not directly impact the performance of each indicator, the doses 25% and 75% of TSE led to best scores for the SPQI (0.71 and 0.70, respectively). By analyzing the scores for the studied functions, there was no difference between TSE doses for f(i), f(ii) and f(iv). However, scores for f(iii) in T1, T3 and T5 were higher than in T2 and T4 (Figure 8), which relates to the overall higher SPQI for T3 and T5 (Figure 7).

Although some works reported that high doses of TSE are beneficial as it increases SOM/SOC (Matheyarasu et al., 2017, Liu and Haynes, 2013; Guo and Sims, 2003; Whells and Whitton, 1969), the highest dose of TSE applied in this study, T2 – 100% TSE, may have resulted in a loss of soil physical quality due to possible pore clogging, which resulted in lower soil macroporosity (Bedbabis et al., 2014; Alves et al., 2015), and, consequently, lower scores for f(iii), soil aeration. Although no statistical differences were found, in absolute terms the lowest macroporosity means were found for T2 – 100% TSE and T4 – 50% TSE.

3.5 Conclusions

Although different doses of treated slaughterhouse effluent (TSE) were applied (T1 – 0% TSE, T2 – 100% TSE, T3 – 75% TSE, T4 – 50% TSE, T5 – 25% TSE), no significant differences were found for the physical indicators of soil quality studied (BD, RP, WFPS, Map, TP, SSI, MWD and Kfs). This suggests that in comparison with T1 – 0% TSE, synthetic fertilizer, there is no negative impacts from TSE on these indicators, making TSE a viable alternative to save fresh water.

SOC was strongly correlated with most physical indicators of soil quality studied, which emphasizes the importance of SOM in agricultural systems. Adding TSE to the soil may increase SOC, leading to an enhancement of soil physical quality. The principal component analysis pointed that BD, RP, SOC and Map were the indicators that most impacted the treatments studied, which clearly separated treatments with TSE from T6 – NV.

By assessing soil quality, the native vegetation was the treatment with higher scores for most of the soil functions studied in relation to other treatments, which resulted in the highest SQPI for the native vegetation. Following T6 – NV, the treatments T3 – 75% TSE and T5 – 25% TSE were the ones with higher SPQI, suggesting that these are the more adequate doses of TSE to improve soil physical quality in the studied conditions. Although no statistical differences were found between physical indicators of soil quality, TSE application to crops may result in an enhanced soil physical quality in adequate doses, taking into consideration possible negative impacts, such as soil salinization and sodification.

In relation to regeneration of agricultural systems, in order to perform as close as to native vegetation areas without losing crop yield, it is necessary to further study the application of agro-industrial effluents, TSE included. Such studies may contribute in future studies to a successful application of circular economy to global agriculture and ensure food security, as alternative sources of food production help to tackle the current crisis in the food and beverage sector.

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4. SOIL CHEMICAL QUALITY IN SYSTEMS IRRIGATED WITH SLAUGHTERHOUSE EFFLUENT

Abstract

In a scenario of climate change and shortage of water and resources necessary as inputs for agriculture, can culminate in a scenario of food insecurity. Thus, the agricultural reuse of agro-industrial effluents, such as slaughterhouse effluent, is an environmentally correct way to preserve ecosystems, contribute to soil health and increase crop productivity. However, very few studies have been developed regarding the impact of applying this effluent on the chemical indicators of soil quality. Thus, this work aimed to evaluate the chemical quality of the soil after receiving irrigation with treated slaughterhouse effluent in an oat-soybean succession system over two years (2020-2022). The experiment was designed in randomized blocks, with five treatments and four replications, namely: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50% and T5 - 25% of the doses of nitrogen (N) recommended for crops applied through irrigation with treated slaughterhouse effluent (TSE). In treatment T1 - 0%, the required N doses were supplied via nitrogen fertilizer in the form of urea, by fertigation. A sixth treatment T6 – NV, was included to compare soil quality indicators, an area of native vegetation (seasonal semideciduous forest). A set of 16 chemical quality indicators was evaluated (macro and micronutrients) and grouped into three subfunctions to compose the Soil Chemical Quality Index (SCQI). The three subfunctions evaluated, Sf(i)- Nutrient availability, Sf(ii) – Acidity and Sf(iii) – Nutrient storage and cycling, compose the main function of Storage, availability and cycling of nutrients. The application of treated slaughterhouse effluent in different doses generally promoted an increase in macro and micronutrients, with negative effects only on potassium levels. The treatments from T1 to T5, differed from T6 (NV) for most of the evaluated indicators, with higher averages verified for T6. Although treatment T3 (75% TSE) was the one that most positively impacted chemical indicators, there were no differences between it and treatments T2 (100% TSE), T4 (50% TSE) and T6 (NV) for SCQI. This indicates that the treatments that received the lowest TSE inputs, in the case of T1 (0% TSE) and T5 (25% TSE), were the ones that presented the lowest SCQI.

Keywords: Soil fertility; Wastewater reuse; Agroindustrial effluent; Soil health.

4.1 Introduction

In a scenario of water shortage, the reuse of effluents such as the slaughterhouse is considered a powerful alternative for water supply and as a source of macronutrients, especially N (nitrogen), P (phosphorus) and K (potassium) (Liu and Haynes, 2013; Menegassi et al., 2020). The benefits of this practice have been proven both in protected environments (Matheyarasu et al., 2016a) and at field levels (Menegassi et al., 2020; Oliveira et al., 2017).

However, irrigation with effluents in general can carry some risks such as salinization, sodification and being a source of pathogens (Lal, 2009; Sandri and Rosa, 2017; Abd-Elwahed, 2018). For this reason, the WHO (2006) effluent reuse guideline gives guidelines on correctly using effluents in agriculture to avoid microbiological and chemical contamination from this practice. Although this practice may still be inadequate for the irrigation of food consumed in natura

(Mcheik et al. 2018), some countries such as Brazil have already created legislation to regulate agricultural reuse in annual crops for human food (CONAMA, 2021).

The slaughter industry demands large volumes of water, which results in an effluent with high BOD, concentration of nutrients, salts, potentially toxic metals and total suspended solids (Bustillo-Lecompte and Mehrvar, 2017; Harris and McCabe, 2015). Therefore, the treatment of these effluents is crucial for activities such as irrigation to avoid these potential negative effects (Bustillo-Lecompte and Mehvar, 2016). From the point of view of soil fertility, some studies have already reported the benefits of treating effluent (Matheyarasu et al., 2017; Guo and Sims, 2003a), but there are still few studies in Brazil on this subject (Menegassi et al., 2020; Luchese et al., 2017; Oliveira et al., 2017a, b).

As Brazil has great representation in world livestock, being the main exporter of beef and poultry (USDA, 2023), giving a correct destination to the effluent generated in this industry is a way to: preserve water resources, reduce costs with synthetic fertilizers and improve soil quality. As soil health, defined as "the ability of the soil to sustain the productivity, diversity, and environmental services of terrestrial ecosystems" (ITPS, 2020), is composed of the chemical, physical and biological spheres, the present work lends itself to evaluating the chemical quality of soils from irrigation with treated slaughterhouse effluent. The specific objectives of this study are: i) to characterize and evaluate the treated effluent from slaughterhouses used in the irrigation of oat/soybean crops; ii) to measure the impact of this irrigation on soil chemical quality indicators and iii) to evaluate the impact of different doses of treated slaughterhouse effluent on the soil chemical quality index (SCQI), in comparison with areas of native vegetation.

4.2 Material and Methods

4.2.1 Study field and experimental design

The experiment was conducted in the municipality of Pirassununga (21°59’ S, 47°26’ W, 635 m asl), São Paulo State, Brazil, in an area adjacent to a slaughterhouse from the Faculty of Animal Science and Food Engineering at the University of São Paulo, “Fernando Costa” campus. The climate in the region is Cwa, humid subtropical with dry winter and hot summer (Alvares et al., 2013), and the average annual temperature is 20.8°C, with an average annual rainfall of 1298 mm. Before developing this experiment, Menegassi et al. (2020) evaluated the cultivation of Coast-cross grass (*Cynodon dactylon* (L.) Pers.) irrigated with treated slaughterhouse effluent (ETA).

Information related to soil type, texture, particle density, soil organic carbon, and land use history is shown in Table 1 and information related to soil chemical attributes is shown in Table 2.

Table 1. Soil classification and soil properties for the experimental area and the soil under native vegetation.

Soil Classification	**Soil Layer**	**Clay**	**Silt**	**Sand**	**PD**	**SOC**	**Drainage**	**Land use change and management**
	cm		g kg^{-1}		g cm^{-3}	g kg^{-1}		
Experimental Area								
Eutric Rhodic Ferralsol	0-10	466	144	390	2.86	13.63	well drained	Conversion to pasture (*Brachiaria decumbens*) and cultivated with coastcross grass (*Cynodon dactylon* (L.) Pers.) from 2017 to 2019 by Menegassi et al. (2020)
	10-20	427	162	411	2.88	13.22		
	20-40	482	118	400	2.88	11.29		
Native vegetation								
Eutric Rhodic Ferralsol	0-40	511	126	363	2.83	15.79	well drained	Semideciduous seasonal forest, part of the ecotone Cerrado-Atlantic Forest

[a] Santos (2018). PD – soil particle density; SOC – soil organic carbon.

Table 2. Soil Chemical attributes for experimental field and native vegetation in the 0-10, 10-20, and 20-40 cm depth.

Experimental field	pH	P	S	K	Ca	Mg	H+Al	SB	CEC	OM	SOC	TN
		mg dm^{-3}				mmol dm^{-3}					g kg^{-1}	
0-10 cm	5,49	22,35	8,35	1,68	37,75	16,20	31,30	55,72	87,05	23,51	13,64	2,24
10-20 cm	5,28	19,20	9,60	1,14	34,05	13,20	34,57	48,47	82,95	22,78	13,22	1,93
20-40 cm	5,13	13,40	8,80	0,68	28,80	8,65	40,12	38,20	78,25	19,47	11,29	1,54

Experimental field	B	Cu	Fe	Mn	Zn	BS	EC
			m dm^{-3}			%	dS m^{-1}
0-10 cm	0,65	9,73	32,25	15,35	4,30	63,53	0,22
10-20 cm	0,61	8,58	29,10	12,34	3,88	58,03	0,19
20-40 cm	0,59	8,49	26,55	8,19	3,04	48,36	0,12

Native vegetation	pH	P	S	K	Ca	Mg	H+Al	BS	CEC	OM	SOC	TN
		m dm^{-3}				mmol dm^{-3}					g kg^{-1}	
0-10 cm	5,03	11,00	12,33	2,30	18,67	9,00	35,97	29,97	66,33	36,07	20,83	2,99
10-20 cm	4,70	10,33	12,00	1,87	17,67	7,00	53,01	26,33	79,67	34,10	19,79	2,24
20-40 cm	4,40	7,00	24,00	1,43	12,67	4,67	70,95	18,80	89,67	26,50	15,41	2,01

Native vegetation	B	Cu	Fe	Mn	Zn	BS	EC
			m dm^{-3}			%	dS m^{-1}
0-10 cm	0,69	5,20	48,00	21,30	2,50	46,30	0,30
10-20 cm	0,37	5,43	40,00	15,83	1,97	34,70	0,31
20-40 cm	0,38	5,40	28,67	9,63	1,47	21,33	0,36

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity.

The experiment was designed in randomized blocks, with five treatments and four replications, namely: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50% and T5 - 25% of the doses of nitrogen (N) recommended for crops applied by irrigation with treated slaughterhouse effluent (TSE). In treatment T1 - 0%, the required N doses were supplied via nitrogen fertilizer in the form of urea, by fertigation. To compare soil quality indicators, a sixth treatment T6 – NV was studied, an area of native vegetation (seasonal semideciduous forest). The study was conducted between 2020 -2022, and a crop rotation of black oat and soybean was established during these two years.

4.2.2 Water source and irrigation characteristic

The effluent used for crop irrigation was provided from the Faculty of Animal Science and Food Engineering slaughterhouse at the University of São Paulo, "Fernando Costa" campus. The generated effluent was preliminarily treated through a solid separation tank, measuring 3.0 m x 3.20 m and 1.20 m in depth. After this process, the effluent was pumped to the UASB (Upflow Anaerobic Sludge Blanket) reactor for treatment. The UASB reactor implanted in an area adjacent to the cultivation area, had a working volume of 12 m^3, with an application rate ranging from 2 to 4 $kg.m^{-3}$ of chemical oxygen demand (COD) as described by Menegassi et al. (2020). The UASB operated in a continuous flow regime, with 24 hours of hydraulic retention. After the anaerobic treatment, the effluent was treated by a polishing pond and pumped into a 5000 L reservoir for irrigation.

Sample collection for effluent analysis was performed weekly according to the National Guide for the Collection and Preservation of Water Samples (CETESB/ANA, 2011) and analyzed according to APHA/AWWA/WEF (2012). The physicochemical characterization of the effluent was performed for electrical conductivity, pH, chemical oxygen demand (COD), total solids (TS), nitrogen series (NTK, NH_4^+, Norg, NO_3^-, NO_2^-, NT), phosphorus (P-PO_4^-), potassium (K^+), sulfate (SO_4^-), calcium (Ca^{+2}), magnesium (Mg^{+2}) and sodium (Na^+). From the concentrations of Ca, Mg and Na, the sodium adsorption ratio (RAS) was calculated by the method described by Ayers & Westcot (1999).

The irrigation system used in the experiment was a conventional sprinkler, with sprinklers located at the two diagonal ends of the experimental plots, with an operating angle of 90º, at an initial height of 1 m from the ground. The sprinkler had an adjustable sectoral impact, with a 3.18 mm nozzle, a flow rate of 0.50 $m^3.h^{-1}$ and a working pressure of 25 mH2O. Christiansen's uniformity test was performed, as described by Bernardo et al. (2006), obtaining the respective mean values for each treatment: T1 = 76%; T2 = 77%; T3 = 77%; T4 = 85%; T5 = 80%. These treatments were individualized by solenoid valves, operated by two control panels responsible for irrigation with water and effluent, aiming at the proposed doses for treatments from T1 to T5. The water reservoir had a storage capacity of 3000 L. The centrifugal motor pump set and PVC tubes were responsible for the flow of water to the sprinkler system.

The irrigation management used aimed to maintain soil water tensions between field capacity and critical tensions for crops in the rotation system in the 0 – 20 cm layer of soil. For this purpose, a capacitive sensor of soil moisture was used in the measurements, through frequency domain reflectometry. The irrigation management frequency adopted was 2 days and the humidity in the capacity of the field, critical and permanent wilting point obtained by the soil water retention characteristic curve (WRC), at a depth of 0 -20 cm, determined by a pressure chamber (Richards, 1941). The adjustments of the WRC parameters were performed using the model of Van Genuchten (1980), through the RETC software (Van Genuchten et al., 2009).

4.2.3 Crops and fertilization

Black oat (*Avena stringosa* Schreb) was sown during the first cultivation cycle on July 30, 2020, with a seeding density of 100 kg ha^{-1}, which emerged on August 6, 2020, and was cultivated by 60 days after sowing (DAS). Soybean (*Glycine max* (L.) Merr.) was sown on October 15, 2020, after dissection of black oat, at a density of 15 seeds per linear meter, which emerged on October 19, 2020. and was cultivated until February 18, 2021 (120 DAS).

After soybean harvesting, a new cycle of black oat cultivation was established in the experimental area, which was sown on May 21, 2021, emerged on May 28, 2021 and cultivated for 110 DAS. Once the oat cycle was over, the second soybean cycle was started on October 29, 2021, which emerged on November 7, 2021, and was carried out until March 3, 2022.

During the first year of black oat cultivation (2020), all treatments got 30 kg ha^{-1} of potassium (K_2O), except T6, and treatment T1 was applied with 80 kg ha^{-1} of N in by urea. These applications were fractionated (four times) during the crop cycle and provided via fertigation. Likewise, in 2021, all treatments received 60 kg ha^{-1} of K_2O and 40 kg ha^{-1} of P_2O_5. Treatment T1 received 60 kg ha^{-1} of N from urea.

Similarly, soybeans received through fertigation 20 kg ha^{-1} of P_2O_5 after the sowing in 2020-2021 and 60 kg ha^{-1} of K_2O during 2020, and 30 kg ha^{-1} of K_2O during 2021. During 2020 and 2021 the following macro-micronutrients were applied: 0.7 kg ha^{-1} of Mn, 2.1 kg ha^{-1} of Zn, 0.046 kg ha^{-1} of B, 1.98 kg ha^{-1} of S, 0.35 kg ha^{-1} of Mg and 3.73 kg ha^{-1} of N.

4.2.4 Soil chemical attributes evaluation

Soil sampling and field assessments were carried out in July 2020 (characterization), March 2021, and March 2022. In each one of the four replicates from systems T1 to T5, three points were chosen to compose one sample, in the interrow, avoiding anthills or compaction zones. In the native vegetation, the soil was similarly sampled, in three fragments of native vegetation, set 5 m apart from each other, avoiding sampling next to anthills, animal burrows, and tall trees, as described by Cherubin et al. (2016).

For each plot, samples were taken in the layers of 0-10, 10-20 and 20-40 cm, totaling 69 points and 69 composed soil samples per year (2021 and 2022). The maximum soil depth of 40 cm was used due to the effective rooting depth of crops in rotational systems (Fan et al., 2016; Myers, 1980). During March 2021, soil samples were also collected at the same scheme before mentioned, for the determination of total Nitrogen during black oat cultivation. Samplings were carried out at

38, 68, and 112 days after sowing (DAS) in May 2021, to understand the dynamics of nitrogen during black oat cultivation.

Macronutrients (except total nitrogen) and micronutrients (available P, K, Ca, Mg, S-SO_4, B, Cu, Fe, Mn, Zn), activity acidity (pH_{CaCl2}), soil organic carbon (SOC), base saturation (SB) and cation exchange capacity (CEC_{pH7}) were determined following the analytical methods described by Raij et al. (2001). Total Nitrogen (TN) and electrical conductivity (EC) were determined by adopting the manual of soil analysis methods, by Teixeira et al. (2017). As proposed by Baldotto et al. (2010), the TN stocks were calculated by multiplying the soil depth (cm), bulk density (g cm^3), and TN content (g kg), and dividing by ten.

4.2.5 Soil Chemical Quality Index (SCQI)

The soil chemical quality index was obtained by selecting indicators related to the function "Storage, availability and cycling of nutrients". For the composition of this function, as adopted by Cherubin et al. (2016), three subfunctions and their associated indicators were considered. For the composition of each of these subfunctions, the indicators were linearly transformed as suggested by Andrews et al. (2002), according to three situations: "more is better", "less is better" and "optimum". For indicators classified as "more is better", each observation was divided by the highest value within the dataset; for "less is better", the lowest value within the dataset was divided for each observation and for "optimum", an ideal value was established, observations were scored either as "more is better" for values lower than the ideal value, and as "less is better" for values higher than the ideal value.

Table 4 presents the subfunctions, indicators, assigned weights, and scoring curves for the composition of the soil chemical quality index (SCQI). It is important to mention that for the selection of these indicators, studies such as those by Barbosa et al. (2018) and Do Carmo Lucio et al. (2014) were also considered.

Table 4. Soil functions and indicators considered for composite the soil chemical quality index (SCQI)

Function	Subfunction	Level 1 Weight	Indicators	Level 2 Weight	Indicators	Level 3 Weight	Scoring curves	Reference
Storage, availability and cycling of nutrients	Sf (i) - Nutrient availability	0.4	Macronutrients	0.8	TN	0.2	more is better	Cherubin et al. (2016)
					P	0.2	more is better	Raij et al. (2001)
					S	0.15	more is better	Raij et al. (2001)
					Ca	0.15	more is better	Raij et al. (2001)
					Mg	0.15	more is better	Raij et al. (2001)
					K	0.15	more is better	Raij et al. (2001)
			Micronutrients	0.2	B	0.2	more is better	Raij et al. (2001)
					Cu	0.2	more is better	Raij et al. (2001)
					Fe	0.2	more is better	Raij et al. (2001)
					Mn	0.2	more is better	Raij et al. (2001)
					Zn	0.2	more is better	Raij et al. (2001)
	Sf (ii) - Acidity	0.4	pH	0.25			optimum	Raij et al. (2001)
			SB	0.25			More is better	Raij et al. (2001)
			HAL	0.50			Less is better	Raij et al. (2001)
	Sf (iii) - Nutrient storage and cycling	0.2	CECpH7	0.4			more is better	Raij et al. (2001)
			SOM	0.6			more is better	Lopes et al. (2013)

The SCQI, was calculated following the equation (1), for all treatments (T1 to T6) and two cultivation years, 2021 and 2022.

$$SCQI = Sf(i) + Sf(ii) + Sf(iii)$$

Where:

$Sf\ (i) = 0.4 * (0.8 * macronutrientes + 0.2 * micronutrients)$

$Sf\ (ii) = 0.4 * (0.25 * pH + 0.25 * (H + Al) + 0.5 * SB)$

$Sf\ (iii) = 0.2 * (0.4 * CEC + 0.6 * SOM)$

4.2.6 Data analyses

The dataset was initially tested for normality by the Shapiro-Wilk test ($p<0.05$) and whenever necessary data was transformed according to Box and Cox (1964). Analysis of variance (ANOVA) was performed ($p<0.05$) and whenever results were significant, they were compared by using regression analysis (treatments T2 to T5) and by analysis of contrast with the Scheffé test ($p<0.05$) (T1 against T2 to T6, and T6 against T1 to T5).

Scores for chemical indicators of soil quality and the soil chemical quality indexes in each treatment were submitted to the Tukey test ($p<0.05$). The Spearman correlation coefficient was also calculated between soil chemical indicators and SCQI (Soil chemical quality index) to analyze the relationship between variables. The statistical analyses were performed using the environment RStudio 1.4.1103 (Rstudio. 2023) and in the SISVAR software (Ferreira, 2011).

4.3 Results

4.3.1 Slaughterhouse effluent, irrigation and nutrient supply

The physicochemical characterization of the slaughterhouse effluent applied during the experiment is shown in Table 5. Treatment T2 – 100% TSE was the one with the highest irrigation depth and TN supply for both crops and cycles (Table 6), followed by treatments T3 – 75%, T4 – 50 % and T5 – 25%, as expected. The total irrigation depth for the period from 2020 to 2022 for two cycles of black oats and two of soybeans is shown in Figure 2. Nutrient supply is shown in Table 6. It is important to note that there was no nitrogen fertilization in the soybean for the treatment 0% TSE. The N supply came from the micronutrient application.

Table 5. Mean values followed by standard deviation for the physico-chemical characterization of the treated slaughterhouse effluent (TSE) and tap water (TW) between 2020 and 2022.

Parameter		**TSE**			**SW**
N-NH_4^+	(mg.L^{-1})	45.81	±	20.18	0.00
N-NTK	(mg.L^{-1})	60.53	±	31.60	1.68
N-NO_3^-	(mg.L^{-1})	1.13	±	0.71	0.80
N-NO_2^-	(mg.L^{-1})	0.13	±	0.14	ND
N-TN	(mg.L^{-1})	61.21	±	31.15	2.48
Ca^{+2}	(mg.L^{-1})	18.19	±	3.82	6.85
Fe^{+2}	(mg.L^{-1})	1.42	±	2.01	ND
Mg^{+2}	(mg.L^{-1})	1.83	±	0.50	0.57
Mn^{+2}	(mg.L^{-1})	0.09	±	0.04	ND
S-SO_4	(mg.L^{-1})	2.55	±	1.86	ND
Na^+	(mg.L^{-1})	34.11	±	9.58	0.80
K^+	(mg.L^{-1})	14.32	±	3.38	0.80
P-PO_4^-	(mg.L^{-1})	5.75	±	2.90	0.08
pH	-	7.87	±	0.66	6.39
EC	(dS m^{-1})	0.55	±	0.17	0.09
SAR	(mmol.L^{-1})$^{-1/2}$	2.11	±	0.68	0.26
COD	(mg.L^{-1})	461.12	±	238.76	-
TS	(mg.L^{-1})	542.08	±	303.08	159.37
TDS	(mg.L^{-1})	218	±	30.47	-

TKN: total Kjeldahl nitrogen; TN: total nitrogen; EC = electrical conductivity; SAR: sodium adsorption ratio; COD: chemical oxygen demand; TS: total solids; TDS: total dissolved solids; ND: Not detectable.

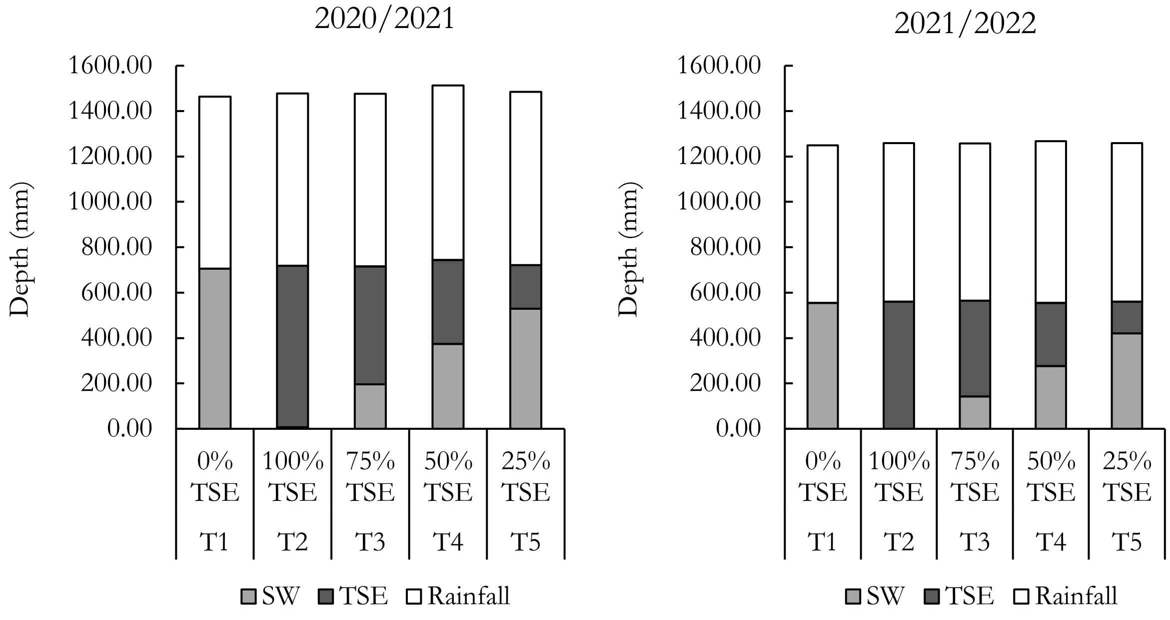

Figure 2. Depth for tap water (TW), treated slaughterhouse effluent (TSE) and rainfall.

Table 6. Supply of macro and micronutrients via treated slaughterhouse effluent (TSE) and conventional fertilization.

Nutrients	TN	Ca	Fe	Mg	Mn	S	Na	K	P	B	Zn
						kg ha^{-1}					
Treatments						Black oat (2020)					
T1	87.34	28.44	0.00	2.38	0.00	0.00	3.32	33.32	0.33	0.00	0.00
T2	411.78	78.82	3.75	6.53	0.25	8.02	154.82	90.57	5.96	0.00	0.00
T3	298.71	56.60	2.70	5.35	0.18	5.76	112.11	89.38	4.37	0.00	0.00
T4	212.39	39.63	1.89	4.48	0.12	4.03	79.51	88.93	3.16	0.00	0.00
T5	123.31	22.14	1.05	3.54	0.07	2.25	45.88	87.72	1.91	0.00	0.00
						Black oat (2021)					
T1	68.90	23.84	0.00	1.99	0.00	0.00	2.78	62.78	40.28	0.00	0.00
T2	188.15	75.09	2.85	7.62	0.42	14.03	97.36	108.78	59.31	0.00	0.00
T3	144.43	62.82	2.15	6.27	0.32	10.60	74.27	97.57	54.66	0.00	0.00
T4	91.52	45.94	1.32	4.47	0.20	6.52	46.51	83.95	49.10	0.00	0.00
T5	52.58	35.99	0.70	3.34	0.10	3.43	25.86	73.98	44.93	0.00	0.00
						Soybean (2020/2021)					
T1	11.17	19.93	0.00	2.02	0.70	1.98	2.33	62.33	20.23	0.05	2.10
T2	120.64	53.59	7.78	5.07	0.87	8.31	106.46	104.37	41.36	0.05	2.10
T3	93.32	45.30	5.83	4.32	0.83	6.73	80.44	93.88	36.08	0.05	2.10
T4	71.31	40.17	4.22	3.84	0.79	5.42	59.06	85.37	31.72	0.05	2.10
T5	39.53	29.42	1.99	2.87	0.74	3.60	29.10	73.20	25.65	0.05	2.10
						Soybean (2021/2022)					
T1	9.02	14.17	0.00	1.54	0.70	1.98	1.65	31.65	20.17	0.05	2.10
T2	168.18	37.38	3.14	4.06	0.88	6.96	71.57	60.09	37.77	0.05	2.10
T3	128.43	31.67	2.36	3.44	0.83	5.72	54.10	53.00	33.37	0.05	2.10
T4	96.21	28.27	1.71	3.03	0.80	4.69	39.88	47.31	29.77	0.05	2.10
T5	50.06	20.76	0.81	2.24	0.75	3.26	19.65	39.02	24.69	0.05	2.10
						Total (2020 - 2021)					
T1	98.52	48.37	0.00	4.40	0.70	1.98	5.65	95.65	20.56	0.05	2.10
T2	532.42	132.41	11.53	11.60	1.12	16.33	261.27	194.95	47.32	0.05	2.10
T3	392.03	101.90	8.53	9.67	1.00	12.49	192.55	183.25	40.45	0.05	2.10
T4	283.70	79.80	6.11	8.32	0.92	9.45	138.58	174.31	34.88	0.05	2.10
T5	162.84	51.56	3.05	6.42	0.81	5.85	74.97	160.92	27.57	0.05	2.10
						Total (2021-2022)					
T1	77.93	38.00	0.00	3.53	0.70	1.98	4.44	94.44	60.44	0.05	2.10
T2	356.33	112.47	5.99	11.68	1.30	20.99	168.93	168.88	97.08	0.05	2.10
T3	272.86	94.50	4.51	9.70	1.15	16.32	128.37	150.57	88.03	0.05	2.10
T4	187.73	74.21	3.03	7.50	0.99	11.21	86.39	131.26	78.86	0.05	2.10
T5	102.64	56.76	1.50	5.58	0.85	6.69	45.51	113.01	69.61	0.05	2.10

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	Total (2020 - 2022)										
	Kg ha^{-1}										
Nutrients	TN	Ca	Fe	Mg	Mn	S	Na	K	P	B	Zn
T1	176.44	86.38	0.00	7.93	1.40	3.96	10.09	190.09	81.01	0.09	4.20
T2	888.75	244.88	17.52	23.28	2.42	37.32	430.20	363.83	144.40	0.09	4.20
T3	664.89	196.40	13.04	19.38	2.16	28.81	320.92	333.82	128.48	0.09	4.20
T4	471.44	154.00	9.14	15.82	1.91	20.66	224.97	305.56	113.75	0.09	4.20
T5	265.48	108.31	4.55	11.99	1.66	12.54	120.48	273.93	97.18	0.09	4.20

TN = total nitrogen, T1 = 0% TSE, T2 =100% TSE, T3 = 75% TSE, T4 = 50% TSE and T5 = 25% TSE

4.3.2 Soil chemical indicators

The application of the treated slaughterhouse effluent showed impacts on the chemical indicators evaluated, except for macronutrients P and S, micronutrients B, Mn, organic matter, soil organic carbon and EC (Figure 3). For these indicators, there was also no interaction between treatment, depth and time. According to the Scheffé test, only for manganese (Mn) there were statistical differences ($p<0.05$) between the treatments T3 to T5 (75, 50 and 25%TSE) and the control T1 (0%TSE), orange line (figure 3), indicated by the hashtag (#). It is important to highlight that sodium is not included in the results, as its concentrations were undetectable although the contribution explained in Table 6 was considerable.

Regarding the pH (Figure 4), the different effluent doses resulted in its increase in a quadratic behavior, being the dose of 50% TSE (T4) the one closest to the maximum point of the curve (5,19). The doses of 25 and 50% TSE differed from the control treatment T1 (0% TSE), being respectively lower and higher than this. As for the macronutrients Ca, Mg and TN similar behavior was verified. For Ca, the dose of 50% TSE (T4) was the closest to the maximum content (32.9 mmol dm^3), since for the dose of 75% TSE (T3) the corresponding content of 33.75 mmol dm^3 is not understood by quadratic regression. For Mg, there was interaction between doses and years, and in 2021 the levels were higher than in 2022. In the year 2021 the dose closest to the maximum point comprised by the quadratic adjustment (15.9 mmol dm^{-3}), was 100%TSE (T2), while for the year 2022 the doses of 50 and 75% TSE (T4 and T3) were the closest to the maximum point (9.83 mmol dm^3). For TN similar behavior was verified (doses of 50 and 75% TSE being the most satisfactory).

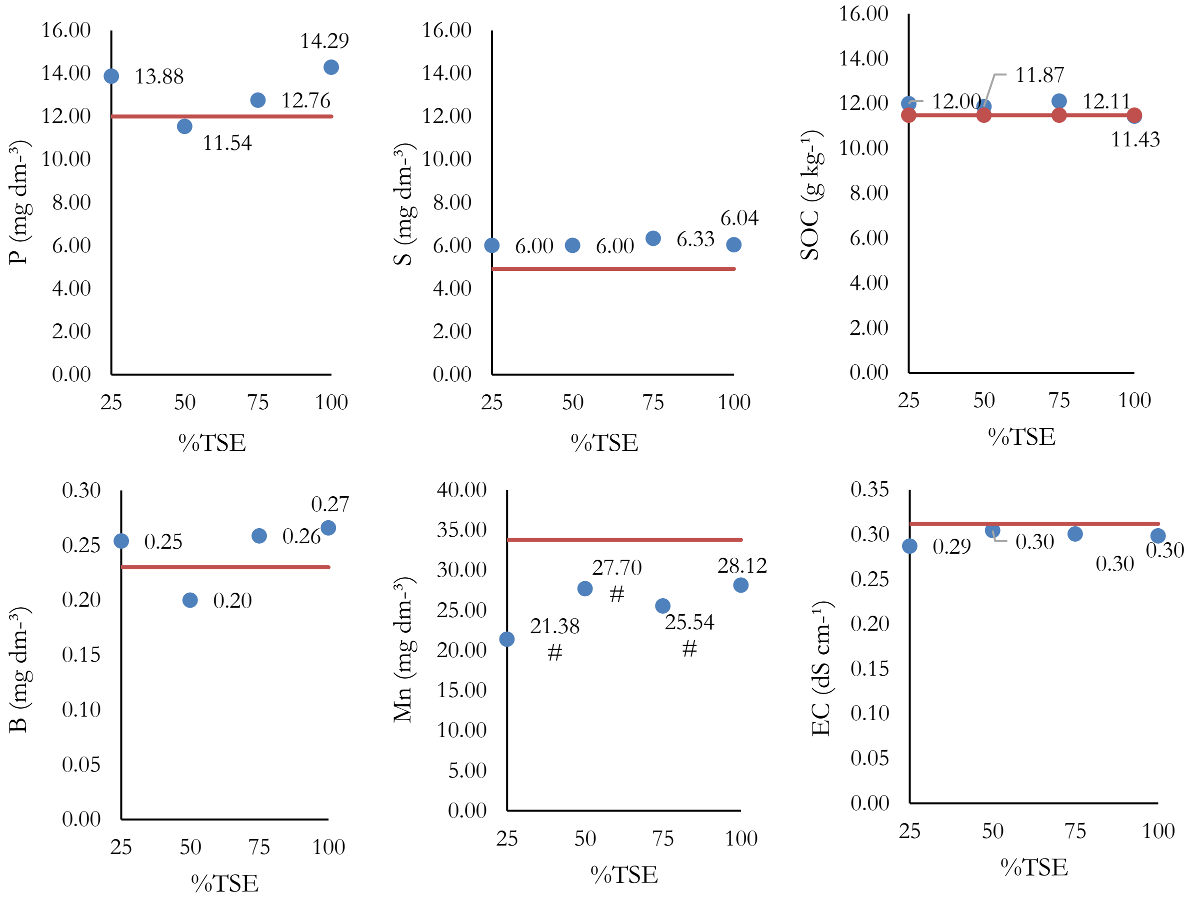

Figure 3. Effect of treatments T2 to T5 (100, 75, 50 and 25% TSE) on phosphorus (P), sulfur (S), soil organic carbon (SOC), boron (B) and manganese (Mn) contents. Means followed by hashtag differ from the T1 treatment (0% TSE – red line) by the Scheffe test ($p<0,05$).

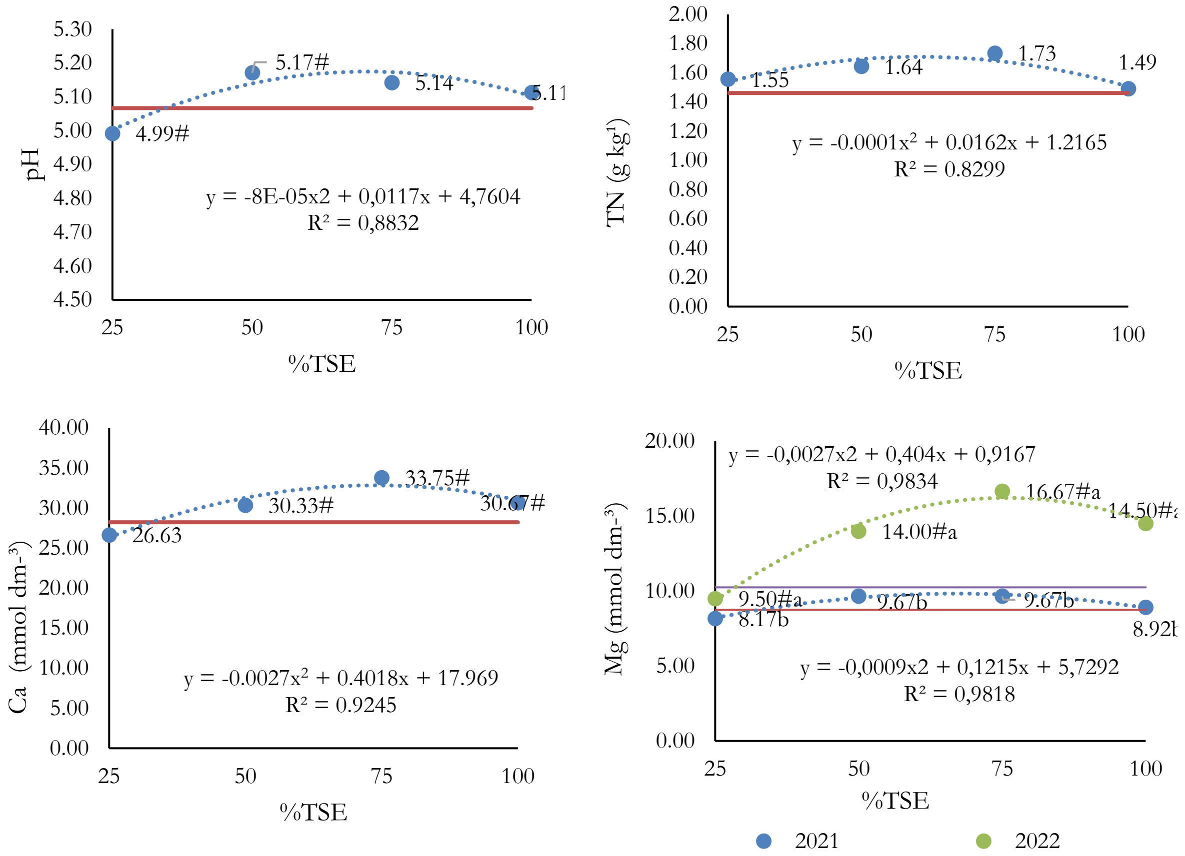

Figure 4. effect of treatments T2 to T5 (100, 75, 50 and 25%TSE) on pH, total nitrogen (TN), soil calcium (Ca) and magnesium (Mg) contents. Means followed by hashtag differ from the T1 treatment (0%TSE) by the Scheffe test (p<0,05).

However, for potassium (K), the increase in effluent doses resulted in a decrease in the contents (linear regression with decreasing behavior) as shown in Figure 5. The doses of 25, 50 and 75% TSE (T4 and T3) differed from the T1 treatment (0% TSE). Similar behavior was verified for the potential acidity (H+Al), being verified in this case interaction between year and treatments (2021 greater than 2022). As for base saturation (BS) and cation exchange capacity (CEC), the doses of 75% TSE and 50% TSE resulted respectively in the highest values associated with these variables, when compared to the maximum points of these regressions (54.9% and 84.9 mmol dm^{-3}). Also, for CEC, the doses of 25, 50, 75 and 100% TSE (T5, T4, T3 and T2) were higher than the T1 treatment (0%TSE).

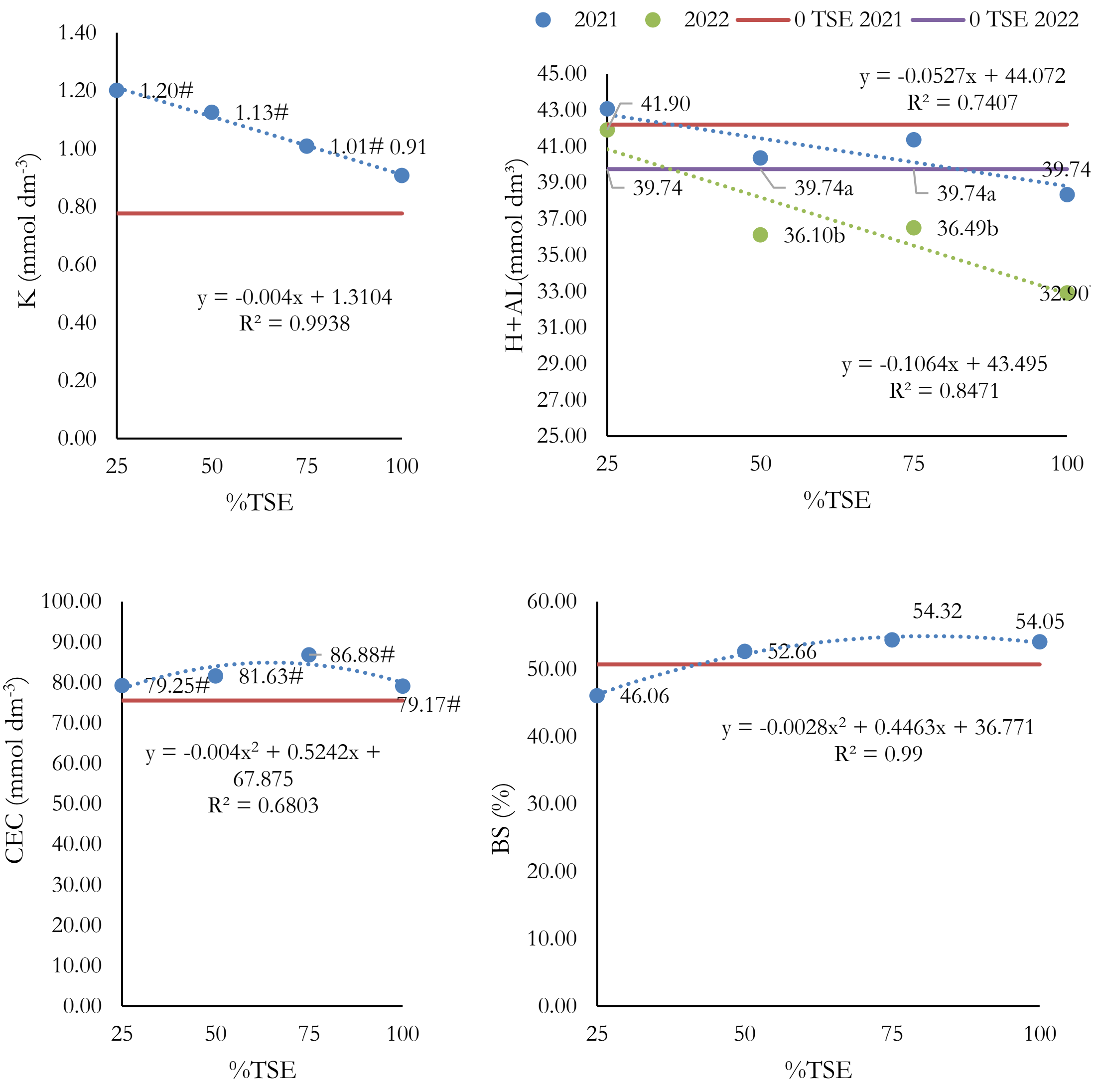

Figure 5. Effect of treatments T2 to T5 (100, 75, 50 and 25%TSE) on potassium (K), active acidity (H+Al), Cation exchange capacity (CEC) and base saturation (BS) contents. Means followed by hashtag differ from the T1 treatment (0%TSE) by the Scheffe test (p<0,05) and different letters indicate differences between the years 2021 and 2022, using the Tukey test (p<0.05).

For the micronutrients, in Fe the dose of 25% TSE was the one that promoted the highest contents (27.88 m dm^{-3}). For Zn, there was interaction between treatments and years, where in 2021 for the doses of 50, 75 and 100% TSE (T4, T3 and T2) the contents were higher than in 2022, and the dose of 100% TSE in both years resulted in the highest values of this micronutrient. In the case of Fe, only the dose of 25% TSE (T5) differed from 0% +TSE (T1), while for Zn no difference was identified. For copper (Cu) the adjustments were linear for treatment x year interaction, and for the doses of 25 and 50% TSE the highest values were found in 2022 and for the doses 75 and 100% TSE the highest values were identified in 2021.

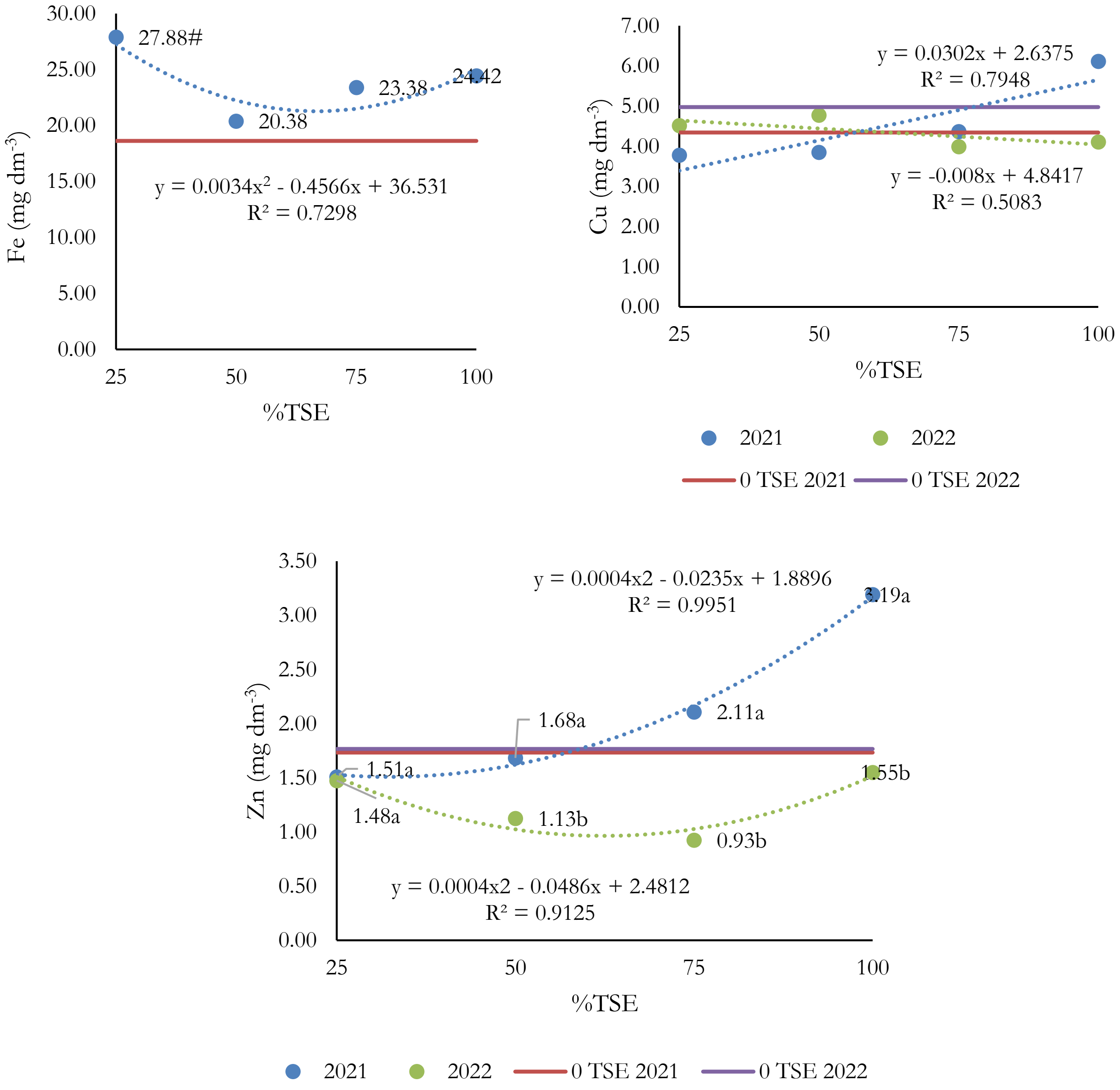

Figure 6. Effect of treatments T2 to T5 (100, 75, 50 and 25% TSE) on iron (Fe), Zinc (Zn) and base Copper (Cu) contents. Means followed by hashtag differ from the T1 treatment (0%TSE) by the Scheffe test ($p<0,05$) and different letters indicate differences between the years 2021 and 2022, using the Tukey test ($p<0.05$).

It is noteworthy that for none of the indicators there was an interaction between the factors treatment and depth. Table 7 presents the statistical differences between the depths of 0-10, 10-20 and 20-40 cm, as well as for the years 2021 and 2022 for the cases where there was no interaction between treatment x year. Except for S and H+Al, the highest levels of macro and micronutrients were found in the 0 – 10 cm layer, and except for P, K, B, Fe, Mn and EC, the values were higher in 2022.

When comparing these same indicators from T1 to T5 (100,75,50 and 25%TSE) with the area of native vegetation (T6), it is possible to identify that in most cases the contrasts were significant (except for P, S, Cu and CE). The pH and Mn for T6 presented the lowest means in relation to the other treatments (4.486 and 16.5 m dm^3 respectively) while for K, OM, SOC, B and H+Al, the means were higher for this treatment in relation to the others (Table 8).

Also for Ca, Mg, Zn, BS, CEC, SB and TN there was interaction between treatments from T1 to T6 and times (2021 and 2022). For Ca and SB, in both years the levels verified for T6 were lower than the other treatments, and in 2022 the levels were higher than in 2021 for the treatments from T1 to T5 (100,75,50 and 25%TSE). Similar behavior was verified for Mg, except for T5 treatment in 2022. For Zn, only the T2 and T3 treatments differed from the T6 treatment in 2021, and these were higher than T2 (100% TSE) and T3 (75% TSE) in 2022 (Table 9).

As for CEC, the highest values were verified for the T6 treatment compared to T1 to T5, and for the year 2022 the values were higher than the year 2021. For TN, the treatments T2, T3 and T4 presented higher averages for 2021 compared to 2022, while for T6 the average of 2.14 g kg in 2022 was higher than 2021 (1.87 g kg). In 2021, only T1 (0%TSE), T2 (100%TSE) and T5 (25%TSE) differed from T6, while in 2022, T1 to T5 were lower than T6.

Table 7. Soil quality chemical indicators in layers of 0-10, 10-20 and 20-40 cm depth, and between years (2021 and 2022).

Depth (cm)	**pH**		**P**		**S**		**K**		**Ca**		**Mg**		**OM**		**B**
			---- m dm^{-3} ----				**--------mmol dm^{-3}--------**						**-g kg^{-1}-**		**m dm^{-3}**
0-10	5.241	a	15.70	a	5.19	b	1.32	a	36.13	a	14.00	a	22.36	a	0.26
10-20	5.109	b	13.68	a	4,78	ab	1.02	b	30.69	b	11.44	b	20.48	b	0.24
20-40	4.963	c	9.97	b	8,31	a	0.84	c	24.22	c	8.72	c	18.46	c	0.23
Depth (cm)	**SOC**		**Fe**		**Mn**		**Zn**		**CEC**		**EC**		**BS**		**H+Al**
	g kg^{-1}		**-------- m dm^{-3}--------**						**mmol dm^{-3}**		**dS m^{-1}**		**%**		**mmol dm^{-3}**
0-10	12.97	a	32.13	a	28.06	a	2.53	a	89.50	a	0.32	a	57.12	a	37.88 b
10-20	11.88	b	28.21	a	24.81	a	1.64	b	82.16	b	0.31	a	52.32	b	38.90 ab
20-40	10.71	c	16.73	b	19.16	b	0.93	c	73.53	c	0.26	b	45.86	c	39.64 ab

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Year	pH	P	S	K	Ca	Mg	OM	B
		---- m dm^{-3}-----			mmol dm^{-3}		--g kg--	m dm^{-3}
2021	5.02 b	14.79 a	3.00 b	1.32 a	26.21 b	9.10 b	19.87 b	0.30 a
2022	5.19 a	11.44 b	9.19 a	0.80 b	34.48 a	13.66 a	20.98 a	0.19 b
	SOC	**Fe**	**Mn**	**Zn**	**CEC**	**EC**	**SB**	**H+Al**
Year	**g kg^{-1}**	**-------- m dm^{-3}--------**			**mmol dm^{-3}**	**dS m^{-1}**	**%**	**mmol dm^{-3}**
2021	11.52 b	28.01 a	27.08 a	1.27 b	77.55 b	0.30 a	47.03 b	43.06 a
2022	12.18 a	23.36 b	21.00 b	2.12 a	85.95 a	0.29 a	56.53 a	41.90 a
	pH	**P**	**S**	**K**	**Ca**	**Mg**	**OM**	**B**
CV 1 (%)	2.24	28.38	74.37	13.07	12.63	15.26	7.47	43.08
CV 2 (%)	2.32	13.72	24.21	20.86	23.04	15.38	4.65	33.34
CV 3 (%)	3.33	34.21	46.72	20.54	14.57	16.28	6.59	48.00
	SOC	**Fe**	**Mn**	**Zn**	**CEC**	**EC**	**BS**	**H+Al**
CV 1 (%)	7.48	33.71	33.71	45.04	6.15	23.99	7.76	4.85
CV 2 (%)	4.66	19.75	19.75	58.51	8.75	65.17	9.91	12.75
CV 3 (%)	6.59	40.28	40.28	48.83	9.09	18.37	10.05	14.47

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity. Means followed by different letters differ by Tukey's test ($p<0.05$).

Table 8. Soil chemical quality indicators for treatments T1 to T5 (0, 100, 75, 50 and 25%TSE) and T6 (Native vegetation), between 2021 and 2022.

Treatment	**pH**	**P**	**S**	**K**	**MO**	**CT**	**B**
		---m dm^{-3}---		**mmol dm^{-3}**	**---g kg^{-1}---**		**m dm^{-3}**
T1 - 0%TSE	5.067 *	12.000	4.917	0.776 *	19.788 *	11.478 *	0.230 *
T2 - 100%TSE	5.135 *	15.800	4.550	0.998 *	20.050 *	11.630 *	0.272 *
T3 - 75%TSE	5.137 *	12.264	6.458	0.962 #*	20.808 *	12.070 *	0.257 *
T4 - 50%TSE	5.171 #*	11.542	6.000	1.125 #*	20.458 *	11.867 *	0.200 *
T5 - 25%TSE	4.992 #*	13.875	6.000	1.201 #*	20.679 *	11.995 *	0.254 *
T6 - NV	**4.486 #**	**10.194**	**17.528**	**2.433 #**	**24.789**	**14.374**	**0.370**
Treatment	**Cu**	**Fe**	**Mn**	**CE**			
		---m dm^{-3}---		**dS m^{-1}**			
T1 - 0%TSE	4.658	18.625	33.792	0.312			
T2 - 100%TSE	5.330	26.500	29.525	0.302			
T3 - 75%TSE	4.124	22.958	25.258	0.298			
T4 - 50%TSE	4.304	20.375	27.704	0.301			
T5 - 25%TSE	4.142	27.875	21.383	0.300			
T6 - NV	**5.736**	**37.556**	**16.539**	**0.297**			

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity. Means followed by hashtag differ from T1, and means followed by asterisk differ from T6 by Scheffe Test ($p<0,05$).

Table 9. Soil chemical quality indicators for treatments T1 to T5 (0, 100, 75, 50 and 25%TSE) and T6 (Native vegetation), between 2021 and 2022.

Treatment	Ca		Mg		Zn		SB	
	--- mmol dm^{-3} ---				--- m dm^{-3} ---		mmol dm^{-3}	
	2021	2022	2021	2022	2021	2022	2021	2022
T1	26.58 *	**29.83** *	8.75 *	**10.25** *	1.73	1.77	36.44 *	40.63 *
T2	26.58 *	**35.15** #*	8.92 *	**14.62** #*	**3.19** #*	1.52	36.79 *	**50.63** #*
T3	27.18 *	**39.75** #*	9.36 *	**16.67** #*	**2.04** *	0.93	37.88 *	**57.33** #*
T4	27.08 *	**33.58** #*	9.67 *	**14.00** #*	1.68	1.13	38.19 *	**48.61** #*
T5	23.42 #*	**29.83** *	8.17 *	9.50 *	1.51	1.48	33.21 *	**40.33** *
T6 – NV	17.00 #	16.67 #	5.00 #	5.94 #	1.31	1.64	24.70	24.87 #

Treatment	CEC		BS		TN	
	mmol dm^{-3}		%		g kg^{-1}	
	2021	2022	2021	2022	2021	2022
T1	76.83 *	74.25 *	47.19 *	**54.16** *	1.46 *	1.46 *
T2	75.25 *	**83.69** #	48.23 *	**60.05** #*	**1.61** *	1.37 *
T3	79.27 *	**93.67** #*	47.53 *	**60.53** #*	**1.87** #	1.57 *
T4	78.50 *	**84.75** #*	48.52 *	**56.80** *	**1.83** #	1.45 *
T5	76.17 *	**82.33** #	43.26 *	**48.86** #*	1.64 *	1.47 *
T6 - NV	84.11	81.33 #	29.39	31.75 #	1.87 #	**2.14**

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity. Means followed by hashtag differ from T1, and means followed by asterisk differ from T6 by Scheffe Test ($p<0,05$). Means in bold are the higher between 2021 and 2022 by the Scheffe test ($p<0,05$).

4.3.3 Total nitrogen on black oat cultivation and TN stocks

As previously reported, total nitrogen during the black oat cycle was evaluated for a better understanding of N dynamics over time. Observing Figure 7, the increment of doses also resulted in a quadratic behavior with interaction in time (38, 68 and 112 DAS).

The highest means for the treatments T5, T4 and T2 (25, 50 and 100% TSE) occurred at 68 and 112 DAS, while for T3 (75% TSE), the highest mean occurred at 112 DAS. At 38 DAS, the T5 treatment (25% TSE) resulted in the highest TN levels (1.92 g kg^{-1}), while at 68 DAS this occurred for T2 (100% TSE) (3.5 g kg^{-1}) and at 112 DAS for T4 (50% TSE) (3.53 g kg-1). Regarding depth, the highest levels were observed for a layer of 0-10 cm on all dates evaluated. When comparing the doses of 25 to 100% TSE as T1 treatment (0% TSE), only the T3 treatment (75% TSE) differed statistically from this, being higher (3.55 g kg^{-1}).

When evaluating the TN stocks in Mg.ha^{-1} at the end of the experiment (2022), there were no differences between the treatments in both years in the 0-40 cm layer evaluated, but the year 2021 presented higher stocks than the year 2022 (Figure 7).

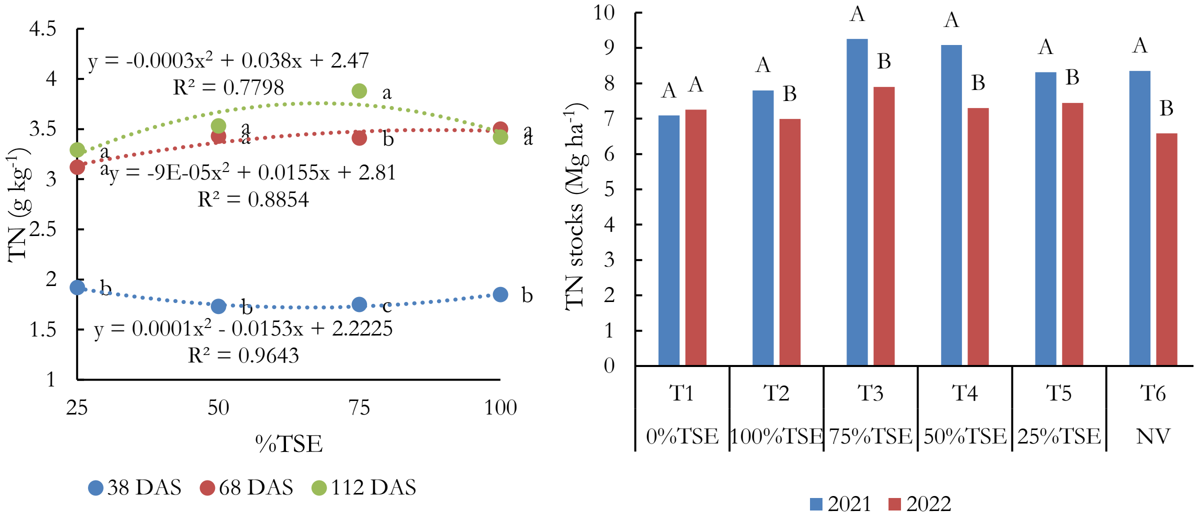

Figure 7. Total Nitrogen (TN) during black oat cultivation in 2021 and Total Nitrogen stocks (0-40 cm) for T1 to T5 (0, 100, 75, 50 and 25% TSE) and T6 (Native vegetation), between 2021 and 2022. Different letters indicate differences between the 38, 68 and 112 days after sowing black oats in 2021, using the Tukey test ($p<0.05$) for the graph on the left. For the straight graph, they indicate differences between the years 2021 and 2022.

4.3.4 Soil chemical quality index and soil chemical indicators correlation

As explained in Figure 8, the application of the doses of 100, 75 and 50% TSE (T2, T3 and T4) resulted in a soil chemical quality index similar to that of the native vegetation area (T6). On the other hand, the doses of 0% TSE (T1) and 25% TSE (T5) were lower than the other treatments. When analyzing the component "subfunctions" of SCQI, there were no differences between treatments for Sf(i) – nutrient availability. For Sf (ii) – acidity, T4 (25% TSE) and T6 (NV) showed the lowest means for SCQI and for Sf (iii) – Nutrient storage and cycling, T6 (NV) was superior to all treatments.

Regarding the correlation between the chemical indicators evaluated and the SCQI, we can observe from Figure 9 that pH was the variable with the strongest correlation with all the variables, presenting positive correlations with Ca, Mg, Mn, Sum of Bases (BS), Base Saturation (SB) and with the SCQI itself. On the other hand, it presented significant negative correlations with S, SOC, B, K, Fe and TN. As expected, CEC, SB, BS, Ca and Mg presented positive correlations with each other, although they presented negative correlations with K.

The electrical conductivity (EC), contents of H+Al, Zn, Cu and P, showed almost no correlations with the other variables (figure 9). For total nitrogen, as expected, there was a positive correlation with the OM and SOC contents, as well as positive correlations with the S, B and Fe contents. Finally, the SCQI was positively and strongly correlated with the contents of Ca, Mg, CEC, BS, also having weaker positive correlations with pH, P, SOC, Mn and SB. The SCQI only showed a negative correlation with the potential acidity (H+Al).

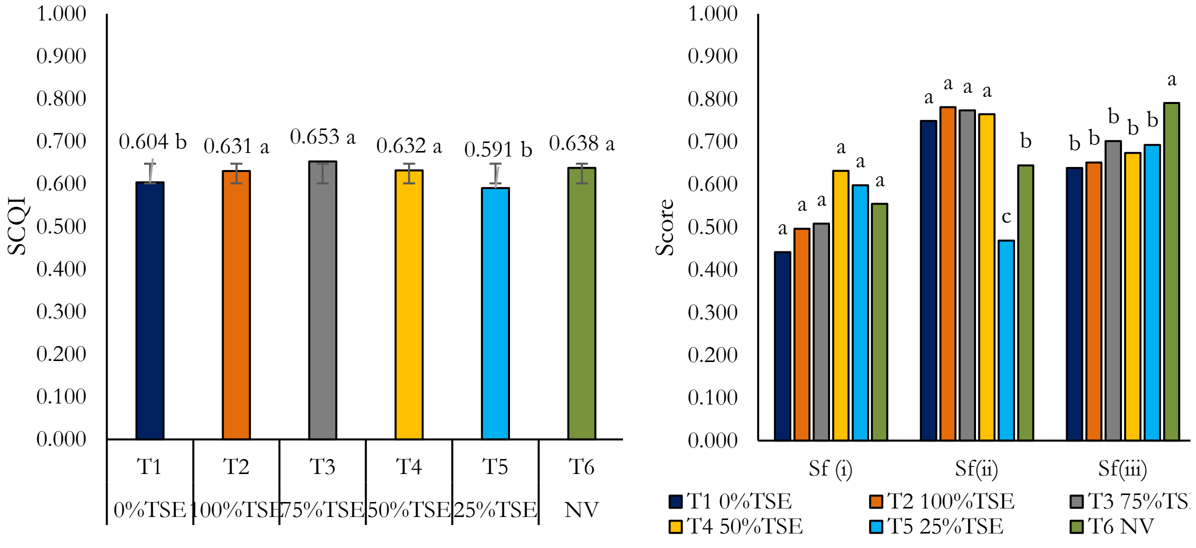

Figure 8. Effects of the application of slaughterhouse effluent, by treatments T1 to T6 (0, 100, 75, 50 and 25% TSE, and NV) on the SCQI - Soil Chemical Quality Index and on the subfunctions Sf (i) - availability of nutrients, Sf (ii) – acidity and Sf (iii) – nutrient storage and cycling. Means followed by different letters differ by the Scott-Knott test ($p<0.10$).

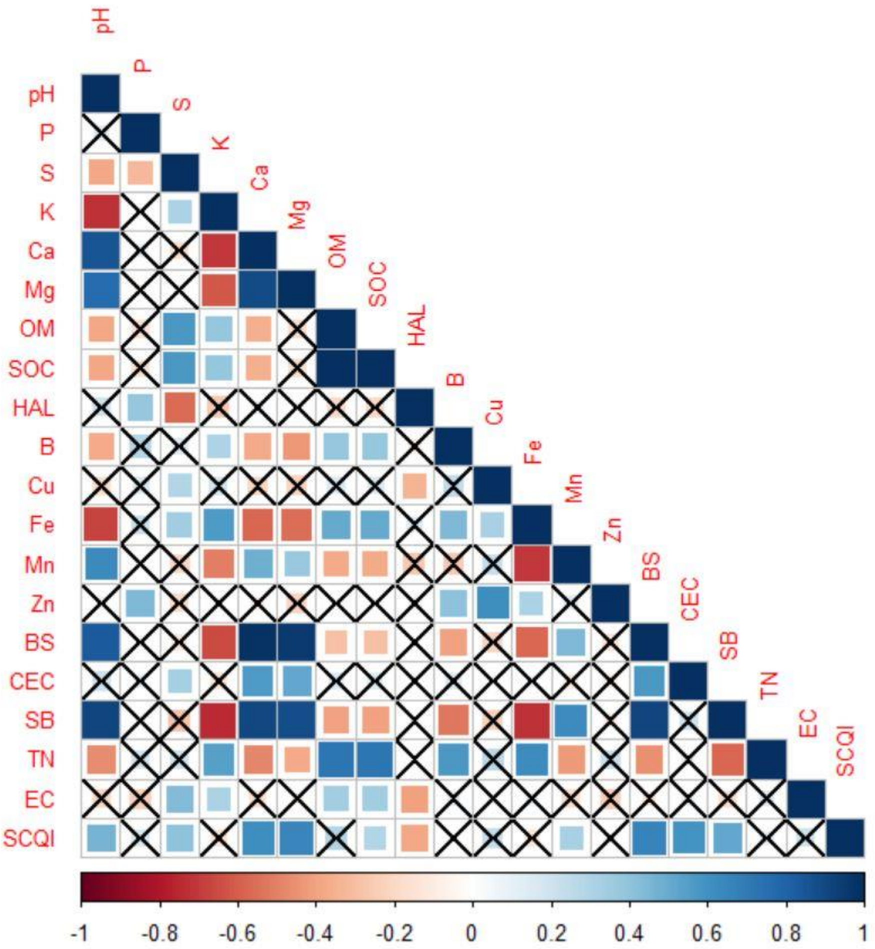

Figure 9. Pearson correlation matrix between chemical indicators of soil quality and SCQI (Soil Chemical Quality Index). Values crossed with an "X" are non-significant ($p<0.05$). P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity.

4.4 Discussion

4.4.1 Slaughterhouse effluent, irrigation and nutrient supply

As shown on Table 5 and according to the resolution number 420 from the Brazilian National Environment Council (Conama, 2011), the effluent would not be suitable for disposal in water bodies as the ammoniacal nitrogen content is over the critical limit (20 mg L^{-1}). However, according to the resolution number 503 (Conama, 2021) regarding the reuse of agro-industrial effluents, the effluent is classified as suitable for agricultural reuse because as the resolution mentions that parameters of agronomic interest are not required to follow the resolution 420 from 2011.

According to Raij et al. (1996), 20 kg ha^{-1} of N is recommended for black oat during sowing and after each cut. In the first cycle of black oat (2020) 80 kg ha^{-1} by means of urea was applied in T1 – 0% TSE, while 60 kg ha^{-1} was applied in the second cycle. It is possible to verify that during the first crop cycle for treatments T2 to T5, nutrient supply was higher than the recommended for fertilization (Table 6). Similarly, nutrient supply in treatments T2 to T4 were higher than the recommended in the second cycle (2021), while it was lower for T5 (52.58 kg ha^{-1}). In relation to soybean, Cordeiro and Echer (2019) suggest a supply of 50 kg ha^{-1} of N combined with biological nitrogen fixation to increase soil N and crop yield. In this view, treatment T5 is within the recommended range. Due to the total irrigation depths being lower in 2021/2022 compared to 2020/2021 (figure 2), the nutrient intake was consequently also low.

4.4.2 Soil chemical indicators

According to Raij et al. (1997), the contents for the macronutrients phosphorus (P), potassium (K) were considered low for the annual crops in question (oats and soybean), in all treatments and in both years (2020/2021 and 2021/2022) (figures 4 and 5, and tables 8 and 9). This may be primarily related to the low P intake via treated slaughterhouse effluent (on average 30 kg ha^{-1}) in each of the oat and soybean cycles. For both oats and soybeans in Brazilian territory, at least fertilizations with 60 kg ha^{-1} and 50 kg ha^{-1} are required for low levels in the soils (7 – 15 m dm-^{3}). Regarding potassium, it may have been leached throughout the experiment due to the high irrigation depths applied.

As for calcium and magnesium, the levels verified for all treatments in both years were high. For sulfur the contents were considered average, as well as pH and base saturation (Raij et al., 1997). For the micronutrients B, Zn, Mn, Cu and Fe, all were in levels considered high, and the addition of effluent doses resulted in the increase of these micronutrients as reported by Matheyarasu et al. (2017 and Seshadri et al. (2014).

When comparing these results with those presented by Menegassi et al. (2020), it is possible to verify great similarity, mainly due to the similar experimental conditions in both studies. In general, the studies that evaluated the fertility of soils after the application of treated effluent from slaughterhouse, indicate increases in phosphorus and potassium contents (Oliveira et al. 2017; Matheyarasu et al., 2017; Matheyarasu et al., 2016; Liu and Haynes, 2013), and also in sodium contents (Menegassi et al., 2020; Oliveira et al., 2017b; Luo et al., 2004). These studies go against the results obtained by the present study, where there was no influence on these nutrients.

The sodium data were not presented in the results because for both years the average contents were practically nil, although table 6 indicates considerable intakes of this element. From these studies, the general impression is that the treated slaughterhouse effluent did not present threats to the salinization and sodification of the soils. This can be endorsed even by the low electrical conductivities for all treatments explained in Figure 3 and in Tables 7, 8 and 9.

Contrary to what is explained by a number of works (Alabi et al., 2019; Da Silva Neto et al., 2013; Guo and Sims, 2003, 2000; Liu and Haynes, 2013; A. V. Luchese et al., 2017; Matheyarasu et al., 2017; Balaji Seshadri et al., 2014), there was an increase in soil pH as the applied effluent doses increased (25, 50, 75 and 100% TSE). These studies report that the low pH of the applied effluent associated with the oxidation of the organic components results in this decrease. However, when evaluating table 5, it is possible to verify that the pH of the effluent applied during the experiment was equal to 7.87, pH considered basic.

Although it is reported in the literature that the application of treated slaughterhouse effluent contributes to increases in organic matter (Liu and Haynes, 2013; Guo and Sims, 2003), there was also no positive impact on this attribute. This may be related to the treatment steps involved in the slaughterhouse effluent used (anaerobic treatment in UASB reactor followed by polishing pond), which efficiently remove organic matter (Vidal et al., 2019; Musa et al., 2019).

The comparison of the treatments T2, T3, T4 and T5 (100, 75, 50 and 25%TSE) with the control treatment T1 (0%TSE), reflect that the addition of all these doses, raise calcium (Ca), magnesium (Mg), sum of bases (SB) and cation exchange capacity (CPB). Although in the case of T1 there were fertilizations with potassium, phosphorus and micronutrients, this treatment differently from the others did not receive constant intakes of calcium and magnesium (Table 6). Thus, it makes sense that it presents lower levels of these macronutrients. Similarly, when comparing the treatments from T1 to T5 with T6 (native vegetation), except for K, OM, SOC and TN (in 2022), the macro and micronutrient contents were higher than the native vegetation due to the constant nutrient intakes via TSE.

4.4.3 Total nitrogen on black oat cultivation and TN stocks

According to Figure 4, the dose that caused the highest levels of TN is 75%TSE (T3), being higher than the control treatment (0%TSE – T1) and equal to the T6 treatment (VN) in 2021 (Table 9). However, in 2022 T6 (NV), presented higher values than the other treatments, with TN stocks being higher in 2021 compared to 2022. This may even be a consequence of the lower contribution between the period of 2021/2022 compared to 2020/2021. When analyzing Figure 4, it is noticed that over an experimental cycle of black oats in 2021, for the T3 treatment (75%TSE), there was an increase in TN levels according to the evaluations in 38, 68 and 112 DAS.

Although irrigation with TSE has led to an increase in TN as doses increase, as reported by other studies (Matheyarasu et al., 2017; Matheyarasu et al., 2016a; Guo and Sims, 2003), it is important to highlight the risks of N losses associated with this management. Nitrogen is very dynamic in soils, and the loss of this via NO, N_2O and N_2 can occur soon after the application of fertilizers, about one day (Dalal et al., 2003). In addition, the treated effluent from the slaughterhouse has high concentrations of nitrogen. The application of this effluent as a source of N and water, causes a permanently humid environment, which can lead to the denitrification process from the reduction of nitrite by ammonia-oxidizing bacteria (Matheyarasu et al., 2016; Wrage-Mönnig et al., 2018; Wrage et al., 2001).

Thus, in 2022 the TN contents were lower than in 2021 possibly both due to the decrease in the contribution of TN as well as due to these losses associated with a permanently irrigated environment.

4.4.4 Soil chemical quality index and soil chemical indicators correlation

As explained in topic 3.3, only the treatments T1 (0% TSE) and T4 (25% TSE) presented indexes lower than the area of native vegetation (T6 – NV). This may be a result of the lower nutrient intake in these treatments, and also in the case of T4, a result of the lower averages presented for pH and SB (indicators related to Sf(ii) – acidity). Although in the case of T6, the acidity is also naturally lower than the other treatments, the organic matter contents are higher than T1, T2, T3, T4 and T5 (Table 8), which raised the SCQI for this treatment. In general, the treatments evaluated suggest a functionality from the point of view of the chemical quality of the soils between 60 and 65%.

As for the correlation matrix (Figure 9), the SCQI showed significant positive correlations with most of the indicators evaluated. However, different from what was expected, it did not present correlations with the total nitrogen and organic matter contents. Much of the TN found in soils is associated with soil organic matter (Leinweber et al., 2013; Nicolás et al., 2018), so these two indicators are strongly correlated (Figure 9). As TSE is a great source of organic matter and nitrogen, it was expected that because SOM plays a central role in soil health (Lal et al., 2016), correlations would be positive between sound and SCQI.

4.5 Conclusions

The application of the treated effluent from the slaughterhouse in different doses, promoted in general the increase of macro and micronutrients, having negative effects only on the potassium contents. Unlike other studies, it also led to an increase in pH and a decrease in potential acidity (H+Al). For most indicators, the effluent dose that caused the highest means was 75% TSE (T3).

Another important conclusion is that the doses of 100, 75 and 50% TSE (T2, T3 and T4) increased the levels of Ca and Mg when compared to the T1 treatment (0%TSE). Consequently, BS, SB and CPB were also elevated in these cases. There was interaction between treatments and evaluation times (2021 and 2022) for Ca, Mg, and SB. BS. CEC, Zn and TN. Only for TN were the levels higher in 2021 when compared to 2022, for all treatments.

The treatments from T1 to T5 differed from T6 (NV) for most of the indicators evaluated, being higher than the means verified for T6. This only did not occur for SOM and TN, where the means were higher for T6 compared to the other treatments. This may be a consequence of the constant contribution of organic matter via litter in the area of native vegetation.

Although the T3 treatment (75% TSE) was the one that most positively impacted the chemical indicators, there were no differences between it and the T2 (100% TSE), T4 (50% TSE) and T6 (NV) treatments for the SCQI. This indicates that the treatments that received the lowest TSE intakes, in the case of T1 (0% TSE) and T5 (25% TSE), were the ones that presented the lowest SCQI.

It is consolidated by the literature that slaughterhouse effluent is a great source of nutrients, mainly N and P, and this work reinforces these findings. What still needs research is how this type of effluent impacts key soil functions over time and how N losses can be decreased.

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5. SOIL QUALITY INDEX APPROACHES IN AN IRRIGATED SYSTEM WITH TREATED SLAUGHTERHOUSE EFFLUENT

Abstract

Amidst climate change, water and resource scarcity, especially for agricultural inputs, can lead to food insecurity. Reusing agroindustrial effluents like slaughterhouse wastewater is an eco-friendly approach to preserving ecosystems, soil health, and boost crop yield. However, few studies have explored its impact on soil quality indicators. This study investigates the effects of irrigating a black oat/soybean succession with treated slaughterhouse effluent (TSE) on soil indicators, comparing it with native vegetation. The experiment, organized in randomized blocks, includes treatments with varying N doses: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50%, and T5 - 25%, applied via irrigation with TSE. Another treatment, T6 - NV, involves an area of native vegetation, for indicator comparison. A total of 26 soil quality indicators were evaluated, which were interpreted using linear and non-linear methods and indexed through different strategies (total dataset - TDS and minimum dataset - MDS). The varying TSE doses (T1 - T5) did not led to statistical distinctions in chemical, physical, and biological indicators, except for differences observed with treatment T6 - NV. Only SQI5, SQI6, and SQI7, assessed through an MDS, exhibited significant differences among treatments, primarily between T6 - NV and others, though not between different TSE doses (except SQI6). These indexes showed the highest sensitivities. Indexes subjected to the same data transformation and indexing method displayed strong positive correlations. However, there was no connection between indexes, carbon stocks (Cstocks), and crop productivity. Future studies should employ an MDS including physical, chemical, and biological indicators to assess the impact of agroindustrial effluents on soil quality. Given the lack of biological indicators from PCA, the SQI7 - SMAF index is recommended as the optimal approach for assessing soil quality.

Keywords: Soil quality indexes; soil functions; SMAF; wastewater reuse.

5.1 Introduction

The population growth over the years has resulted in increased pressure for water resources (Schewe et al., 2014). Alongside, other sectors such as agriculture compete for these resources, with 70% of world water consumption being used for irrigation (WWAP, 2017; Hanjra and Qureshi, 2010). Furthermore, the latest climate crises have changed precipitation patterns worldwide, leading to the worsening of water scarcity in arid zones (Schewe et al., 2014; Hanjra and Qureshi, 2010). In this way, the application of wastewater in agricultural fields emerges as an alternative to reduce freshwater consumption in agriculture, providing nutrients, reducing the need for synthetic fertilizers and constituting an environmentally friendly solution (Fito and Van Hulle, 2020; Helmecke et al., 2020).

The slaughter industry demands high water consumption (Harvey et al., 2017) and the effluents generated have high concentrations of nutrients (N, P, K), organic matter, fats, biochemical oxygen demand (BOD), total suspended solids (TSS) and salts substances such as ammonia, potentially toxic metals (PTM) and pathogens (Bustillo-Lecompte and Mehrvar, 2017; Harris and McCabe, 2015). These characteristics configure the potential pollutant of these wastewaters, but if treated correctly it can be a viable alternative to reduce costs with fertilizers in agriculture and provide an appropriate disposal for these effluents (Vergine et al., 2017; Menegassi et al., 2020).

To assess the advantages of wastewater reuse, closely monitoring soil quality (soil health) becomes crucial. This is because effective soil management enhances the operational capacity of soil-based ecosystem services, leading to enhanced food security through satisfactory crop yields (Bünemann et al., 2018; Hurni et al., 2015; McBratney et al., 2014). Soil quality is defined as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). Studies have shown that the application of TSE (treated slaughterhouse effluent) is beneficial for soil fertility but poses risks such as salinization and sodification (Guo and Sims, 2003a; Matheyarasu et al., 2016a; Menegassi et al., 2020; Osemwota, 2010a). However, there is a lack of studies about wastewater irrigation specifically with slaughterhouse effluent and its impact on soil quality.

The use of indexes to study soil quality constitutes a strategy that helps decision-making in the field (Andrews et al., 2002). However, there are a series of possibilities to compose and constitute them: performing linear or non-linear data transformation (Zhou et al., 2020; Nabiohalli et al., 2018); using a total set of indicators (TDS) or a minimum set (MDS) and assigning weights or not to each of the evaluated indicators (Cherubin et. al., 2016a), using soil functions for example (Vogel et al., 2019). Specifically for studies with the reuse of effluents in agriculture and their impacts on QS, soil functions were studied by Barbosa et al. (2018) to understand how domestic sewage could impact the soil health in sugarcane production areas. Similarly, Rezapour et al. (2021) pursued a comparable comprehension using diverse indexing approaches (MDS and TDS).

Based on these considerations, the objectives of this work were: a) to investigate the impacts of irrigation with TSE in the black oat/soybean succession on the physical, chemical and biological indicators of the soil and its comparison with an area of native vegetation, b) to analyze how to different indexing strategies of the QS indicators behave through the different TSE doses applied and c) to evaluate the correlation of these indexes with the black oat and soybean yields.

5.2 Material and Methods

5.2.1 Study field and experimental design

The experiment was conducted in the municipality of Pirassununga (21°59' S, 47°26' W, 635 m asl), São Paulo State, Brazil, in an area adjacent to the slaughterhouse from the Faculty of Animal Science and Food Engineering at the University of São Paulo, "Fernando Costa" campus. The climate in the region is Cwa, humid subtropical with dry winter and hot summer (Alvares et al., 2013), and the average annual temperature is 20.8°C, with an average annual rainfall of 1298 mm. Before this experiment, Menegassi et al. (2020) evaluated the cultivation of Coast-cross grass (*Cynodon dactylon* (L.) Pers.) irrigated with treated slaughterhouse effluent (ETA). Information related to soil type, texture, particle density, soil organic carbon, and land use history is shown in Table 1 and information related to soil chemical and physical attributes is shown in Tables 2 and 3.

The experiment designed was the randomized blocks, with five treatments and four replications, namely: T1 - 0%, T2 - 100%, T3 - 75%, T4 - 50% and T5 - 25% of the doses of nitrogen (N) recommended for crops applied by irrigation with treated slaughterhouse effluent (TSE). In treatment T1 - 0%, the required N doses were supplied via nitrogen fertilizer in the form of urea, by fertigation. For comparison of soil quality indicators, a sixth treatment T6 – NV, was studied, an area of native vegetation (seasonal semideciduous forest). The study was conducted between 2020 - 2022, and a crop succession of black oat and soybean was maintained during these two years.

Table 1. Soil classification and soil properties for the experimental area and the soil under native vegetation.

Soil Classification	Soil Layer	Clay	Silt	Sand	PD	SOC	Drainage	Land use change and management
	cm		g kg^{-1}		g cm^{-3}	g kg^{-1}		
Experimental Area								
Eutric Rhodic Ferralsol	0-10	466	144	390	2.86	13.63	well drained	Conversion to pasture (*Brachiaria decumbens*) and cultivated with coastcross grass (*Cynodon dactylon* (L.) Pers.) from 2017 to 2019 by Menegassi et al. (2020)
	10-20	427	162	411	2.88	13.22		
	20-40	482	118	400	2.88	11.29		
Native vegetation								
Eutric Rhodic Ferralsol	0-40	511	126	363	2.83	15.79	well drained	Semideciduous seasonal forest, part of the ecotone Cerrado-Atlantic Forest

[a]Santos (2018). PD – soil particle density; SOC – soil organic carbon.

Table 2. Initial soil chemical attributes characterization (June 2020) for experimental field and native vegetation in the 0-10, 10-20, and 20-40 cm depth.

Experimental field	**pH**	**P**	**S**	**K**	**Ca**	**Mg**	**H+Al**	**SB**	**CEC**	**OM**	**SOC**	**TN**
		m dm^{-3}				mmol dm^{-3}					g kg^{-1}	
0-10 cm	5.49	22.35	8.35	1.68	37.75	16.20	31.30	55.72	87.05	23.51	13.64	2.24
10-20 cm	5.28	19.20	9.60	1.14	34.05	13.20	34.57	48.47	82.95	22.78	13.22	1.93
20-40 cm	5.13	1340	8.80	0.68	28.80	8.65	40.12	38.20	78.25	19.47	11.29	1.54

Experimental field	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**BS**	**EC**
			m dm^{-3}			%	dS m^{-1}
0-10 cm	0.65	9.73	32.25	15.35	4.30	63.53	0.22
10-20 cm	0.61	8.58	29.10	12.34	3.88	58.03	0.19
20-40 cm	0.59	8.49	26.55	8.19	3.04	48.36	0.12

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Native vegetation	**pH**	**P**	**S**	**K**	**Ca**	**Mg**	**H+Al**	**BS**	**CEC**	**OM**	**SOC**	**TN**
		m dm^{-3}				mmol dm^{-3}					g kg^{-1}	
0-10 cm	5.03	11.00	12.33	2.30	18.67	9.00	35.97	29.97	66.33	36.07	20.83	2.99
10-20 cm	4.70	10.33	12.00	1.87	17.67	7.00	53.01	26.33	79.67	34.10	19.79	2.24
20-40 cm	4.40	7.00	24.00	1.43	12.67	4.67	70.95	18.80	89.67	26.50	15.41	2.01

Native vegetation	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**BS**	**EC**
			m dm^{-3}			%	dS m^{-1}
0-10 cm	0.69	5.20	48.00	21.30	2.50	46.30	0.30
10-20 cm	0.37	5.43	40.00	15.83	1.97	34.70	0.31
20-40 cm	0.38	5.40	28.67	9.63	1.47	21.33	0.36

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity.

Table 3. Initial soil physical attributes characterization (June 2020) for experimental field and native vegetation in the 0-10, 10-20, and 20-40 cm depth.

Location	Depth (cm)	BD g cm^{-3}	RP MPa	WFPS m^3.m^{-3}	Map m^3.m^{-3}	TP m^3.m^{-3}	SSI %	MWD mm
Experimental field	0-10	1.19	1.17	0.595	0.239	0.587	3.92	4.41
Native vegetation	0-10	1.01	0.98	0.540	0.298	0.648	4.93	4.39
Experimental field	10-20	1.32	1.65	0.726	0.150	0.540	3.80	4.21
Native vegetation	10-20	1.09	1.80	0.580	0.261	0.617	4.76	3.92
Experimental field	20-40	1.31	1.72	0.709	0.160	0.545	3.25	3.75
Native vegetation	20-40	1.03	1.73	0.573	0.274	0.640	5.47	3.95

BD: Bulk density; RP: soil resistance to penetration; Kfs: Field-saturated hydraulic conductivity; WFPS: water filled pore space; Map: Macroporosity; TP: total porosity; MWD: mean weight diameter of soil aggregates; SSI: Stability structural index; SOC: soil organic carbon.

5.2.2 Water source and irrigation characteristics

The effluent used for crop irrigation comes from the Faculty of Animal Science and Food Engineering slaughterhouse at the University of São Paulo, "Fernando Costa" campus. The slaughterhouse effluent was preliminary treated through a solid separation tank, measuring 3.0 m x 3.20 m and 1.20 m in depth. Afterward, the effluent was pumped to the UASB (Upflow Anaerobic Sludge Blanket) reactor for treatment. The UASB reactor located in an area adjacent to the cultivation area, had a work volume of 12 m^3, with an application rate ranging from 2 to 4 kg.m^{-3} of chemical oxygen demand (COD) as described by Menegassi et al. (2020). The UASB operated in a continuous flow regime, with 24 hours of hydraulic retention. After the anaerobic treatment, the effluent was treated by a polishing pond and pumped into a 5000 L reservoir for irrigation.

Samples for effluent analysis were collected weekly according to the National Guide for the Collection and Preservation of Water Samples (CETESB/ANA, 2011) and analyzed according to APHA/AWWA/WEF (2012). The physicochemical characterization of the effluent was performed for electrical conductivity, pH, chemical oxygen demand (COD), total solids (TS), nitrogen series (NTK, NH_4^+, Norg, NO_3^-, NO_2^-, NT), phosphorus (P-PO_4^-), potassium (K^+), sulfate (SO_4^-), calcium (Ca^{+2}), magnesium (Mg^{+2}) and sodium (Na^+). From the concentrations of Ca, Mg and Na, sodium adsorption ratio (RAS) was calculated by the method described by Ayers & Westcot (1999).

The irrigation system used in the experiment was the conventional sprinkler, with sprinklers located at the two diagonal ends of the experimental plots, with an operating angle of 90º, at a height of 1 m from the ground. The sprinkler had an adjustable sectoral impact, with a 3.18 mm nozzle, a flow rate of 0.50 $m^3.h^{-1}$ and a working pressure of 25 mH2O. Christiansen's uniformity test was performed, as described by Bernardo et al. (2006), resulting in the respective mean values for each treatment: T1 = 76%; T2 = 77%; T3 = 77%; T4 = 85%; T5 = 80%. These treatments were individualized by solenoid valves, operated by two control panels responsible for irrigation with water and effluent, aiming at the proposed doses for the treatments from T1 to T5. The water reservoir had a storage capacity of 3000 L. The centrifugal motor pump set and PVC tubes were responsible for the flow of water to the sprinkler system.

The irrigation management aimed to maintain the water tensions between field capacity and critical tensions for crops in the rotation system in the 0 – 20 cm layer of the soil. For this purpose, a capacitive sensor of soil moisture was used in the measurements, through frequency domain reflectometry. The irrigation management frequency adopted was 2 days and the humidity in the field capacity, critical and permanent wilting point obtained by the soil water retention characteristic curve (WRC), at a depth of 0 -20 cm, determined by a pressure chamber (Richards, 1941). The adjustments of the WRC parameters were performed using the model of Van Genuchten (1980), through the RETC software (Van Genuchten et al., 1991).

5.2.3 Crops and fertilization

Black oat (*Avena stringosa* Schreb) was sown during the first cultivation cycle on July 30, 2020, with a seeding density of 100 kg ha^{-1}, which emerged on August 6, 2020, and was cultivated by 60 days after sowing (DAS). Soybean (*Glycine max* (L.) Merr.) was sown on October 15, 2020, after dissection of black oat, at a density of 15 seeds per linear meter, which emerged on October 19, 2020 and was cultivated until February 18, 2021 (120 DAS).

After soybean harvesting, a new cycle of black oat cultivation was established in the experimental area, which was sown on May 21, 2021, emerged on May 28, 2021 and was cultivated for 110 DAS. Once the oat cycle had finished, the second soybean cycle was started on October 29, 2021, which emerged on November 7, 2021 and was conducted until March 3, 2022.

During the first year of black oat cultivation (2020), all treatments got 30 kg ha^{-1} of potassium (K_2O), except T6, and treatment T1 was applied with 80 kg ha^{-1} in from urea. These applications were fractionated during the crop cycle and provided via fertigation. Likewise, in 2021, all treatments received 60 kg ha^{-1} of K_2O and 40 kg ha^{-1} of P_2O_5. Treatment T1 received 60 kg ha^{-1} of N from urea.

Similarly, the soybean received through fertigation 20 kg ha^{-1} of P_2O_5 after the sowing in 2020-2021 and 60 kg ha^{-1} of K_2O during 2020, and 30 kg ha^{-1} of K_2O during 2021. During 2020 and 2021 the following macro-micronutrients were applied: 0.7 kg ha^{-1} of Mn, 2.1 kg ha^{-1} of Zn, 0.046 kg ha^{-1} of B, 1.98 kg ha^{-1} of S, 0.35 kg ha^{-1} of Mg and 3.73 kg ha^{-1} of N.

5.2.4 Soil sampling and analysis

Soil sampling and field assessments were carried out in July 2020 (characterization) and March 2022. In each one of the four replicates from systems T1 to T5, one sampling point was chosen in the center of each plot, in the interrow, avoiding anthills or compaction zones. In the native vegetation, the soil was similarly sampled, in three fragments of native vegetation, set 5 m apart from each other, avoiding sampling next to anthills, animal burrows, and tall trees, as described by Cherubin et al. (2016). For each sampling point, a small trench of 30 x 30 x 40 cm was dug, and samples were taken in the layers of 0-10, 10-20 and 20-40 cm, totaling 23 sampling points and 69 undisturbed soil samples for physical analysis and 69 disturbed samples for chemical/biological analysis. The maximum soil depth of 40 cm was used due to the effective rooting depth of crops in rotational systems (Fan et al., 2016; Myers, 1980).

Macronutrients (except total nitrogen) and micronutrients (available P, K, Ca, Mg, $S\text{-}SO_4$, B, Cu, Fe, Mn, Zn), activity acidity (pH_{CaCl2}), soil organic carbon (SOC), base saturation (SB) and cation exchange capacity (CEC_{pH7}) were determined following the analytical methods described by Raij et al. (2001). Total Nitrogen (TN) and electrical conductivity (EC) were determined by adopting the manual of soil analysis methods, by Teixeira et al. (2017). The enzymatic activity of β-glucosidase was measured following the methodology described by Tabatai (1994).

The undisturbed soil samples from soil cores were used to determine a range of physical indicators of soil quality. The cores were saturated with water and soil microporosity was then determined as the water content at -6 KPa. Soil bulk density (BD, g cm$^{-3)}$ was determined as a fraction between the weight of the soil dried for 48 h at 105°C and the core volume, which was about 97 cm^3 (Grossman and Reinsch, 2002). From soil bulk density and particle density values, it was possible to calculate total porosity (TP, m^3.m^{-3}) as TP = 1 – (BD/PD). Soil macroporosity (Map, m^3.m^{-3}), was determined by subtracting the saturated water content by field capacity (-6 KPa). Water-filled pore space (WFPS) was assessed as the relation between the volumetric water content at field capacity (-6 KPa) and total porosity, as described by Wienhold et al. (2009).

Soil resistance to penetration (SRP, MPa) measurements were carried out in five replicates in each experimental plot and the three fragments of native vegetation by using a digital penetrometer (Penetrolog). Such measurements were performed as close as possible to field capacity, in which the gravimetric water content was constantly monitored. Saturated soil hydraulic conductivity (Kfs, mm.h^{-1}) was measured on-site using the method BEST - Beerkan Estimation of Soil Transfer, as described by Lassabatère et al. (2006). To do so, a steel cylinder (7 cm height, 16 cm diameter) was placed 1 cm down into the soil and 150 mL of water was added in each run eight times, or up to the number of times needed for the infiltration rate to reach a steady state. Saturated soil hydraulic conductivity was thereafter estimated by using the algorithm proposed by Bagarello et al. (2014). This procedure was performed in duplicates in the experimental plots, and the average value was used in this work.

The mean weighted diameter of water-stable aggregates (MWD, mm) was determined according to van Bavel (1950). Undisturbed soil samples of 10 cm^3 were sieved through a 9520 μm sieve and saturated for 24h. The aggregates were thereafter placed at the top of a set of three sieves, 2000, 250 and 53 μm in an apparatus for vertical oscillation at 42 rpm by 15 min (Yoder, 1936). The mean weight diameter was calculated as the weighted sum of the occurrence of each class of aggregate size.

Carbon stocks were calculated using equation 1, following the described by Fernandes and Fernandes (2013) and Carvalho et al (2009). Cstock is the total organic C content at the sampled depth (g kg^{-1}), Bd is the bulk density at the depth sampled (Kg.dm^{-3}), Bdref is the bulk density for the sampled depth in the reference area and “e” is the layer thickness considered.

$$Cstock = \frac{Cs * Bd * \left(\frac{Bdref}{Bd}\right) * e}{10} \qquad (1)$$

5.2.5 Soil quality indexes strategies (SQI)

Different strategies were adopted for measuring soil quality indexes, according to the scheme represented in Figure 1. In total, seven indexes were evaluated, comparing methods of transforming the indicators (linear technique and scoring curves, as proposed by Andrews et al. (2002) and Andrews et al. (2004)) and the indicators integration into a single index (Soil functions, Principal component analysis, weighted additive and Soil Assessment Management Framework - SMAF tool). For all cases, data from the indicators in the 0-10, 10-20 and 20-40 cm layers were averaged to construct a soil quality index (SQI) for the 0-40 cm layer. In both cases of transformation (linear and non-linear) of the indicators between 0 and 1, three cases followed: "more is better", "less is better" and "optimum". For the linear transformations, the following equations were adopted:

$$Score\ (more\ is\ better) = \frac{indicator\ value}{highest\ value\ of\ the\ dataset} \qquad (2)$$

$$Score\ (less\ is\ better) = \frac{lowest\ value\ of\ the\ dataset}{indicator\ value} \qquad (3)$$

For indicators such as pH, an optimum point was adopted as a threshold. For values below the optimal point, the Eq 2 was adopted, while for the values above the optimum point, the Eq 3 was used. For nonlinear transformations, the Equations 4, 5, 6 and 7 were adopted following the cases “more is better, “less is better” and “mid-point optimum” respectively. The Equation 3 represents an upper asymptote sigmoid curve, the Equation 4 a lower asymptote sigmoid curve and the Equations 5 and 6, a Gaussian curve (Cherubin et al., 2016; Silva-Olaya et al., 2022). In these curves LB is the lower baseline value, UB the upper baseline value, LT the lower threshold, UT the upper threshold, x is the indicator value, s is the slope of the equation equals to -2,5 according to Cherubin et al. (2016a) and Silva-Olaya et al. (2022) and O is the optimum point. The supplementary Table 1 (appendix C) shows the parameters adopted in each case of the scoring curves.

$$Score\ (more\ is\ better\ non\ linear) = \frac{1}{1+\left(\frac{LB-UT}{x-UT}\right)^{s}} \qquad (4)$$

$$Score\ (less\ is\ better\ non\ linear) = \frac{1}{1+\left(\frac{LB-LT}{x-LT}\right)^{s}} \qquad (5)$$

$$Score\ (gaussian\ curve\ left\ side) = \frac{1}{1+\left(\frac{LB-O}{x-O}\right)^{S}} \qquad (6)$$

$$Score\ (gaussian\ curve\ right\ side) = \frac{1}{1+\left(\frac{UB-O}{x-O}\right)^{S}} \qquad (7)$$

To evaluate the best strategy to be adopted, these indicators were correlated with the productivity of black oats and soybean considering the results from the second cycle (2021/2022), following Pearson's correlation. The sensitivity of the soil quality indexes was evaluated according to Masto et al. (2008) and Cherubin et al. (2016a), where Sensitivity (S) = SQImax/SQImin.

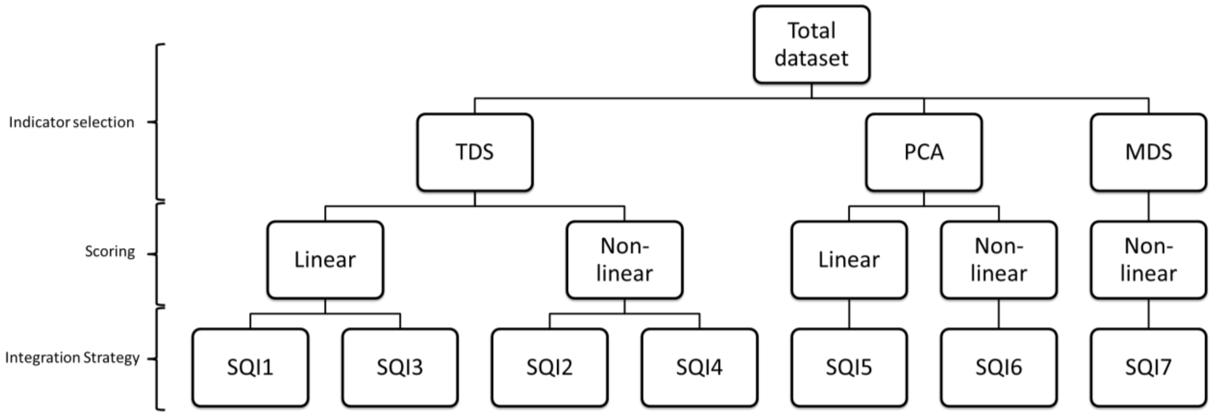

Figure 1: Strategies for indicators selection, scoring and integration to construct de soil quality indexes. TDS: total dataset; PCA: principal component analysis; MDS: minimum dataset; SQI1 – Soil quality index based on soil functions with linear transformation; SQI2 - Soil quality index based on soil functions with non-linear transformation; SQI3 – Soil quality index based on weight additive with linear transformation; SQI4 - Soil quality index based on soil functions with non-linear transformation; SQI5 – Soil quality index based on PCA analysis with linear transformation; SQI6 - Soil quality index based on PCA analysis with non-linear transformation and SQI7 – Soil quality index based on SMAF tool.

5.2.5.1 Soil functions

In this approach, five soil functions were adopted (Cherubin et al., 2016a; Lima et al., 2013), with associated quality indicators: F(i) - Storage, availability and cycling of nutrients, F(ii) - Infiltration, storage and availability of water and soil aeration, F(iii) - Sustain biological activity, F(iv) - Sustain plant growth and F(v) - Ability to resist to degradation. All these functions received the same weight in the final composition of the soil quality index, and the weights of the indicators associated with each of them are described in Table 4. From this approach, the SQI 1 and SQI 2 indicators were derived, where in the first case the data were transformed following equations 2 and 3 and for the second case following equations 4, 5, 6, and 7. The SQI1 and SQI 2 were obtained as described in Eq 8, where wf is the weight of the soil function, wi is the weight of the indicator and si is the score of the indicator.

$$\boldsymbol{SQI\,1\ and\ SQI2 = \sum Wf.Wi.si} \qquad (8)$$

Table 4. Soil functions and indicators related to the SQI 1 and SQI 2.

Soil Functions		**Weight**		**indicators**	**Weight**
F(i)	**Storage, availability and cycling of nutrients**	**0.2**	0.4	**Nutrient availability**	
				Macronutrients	0.8
				TN	0.2
				P	0.2
				K	0.15
				Ca	0.15
				Mg	0.15
				S	0.15
				Micronutrients	0.2
				B	0.2
				Cu	0.2
				Mn	0.2
				Fe	0.2
				Zn	0.2
			0.4	**Acidity**	
				pH	0.25
				H+Al	0.25
				BS	0.5
			0.15	**Nutrient storage and cycling**	
				CEC	0.4
				SOC	0.6
			0.05	**Nutrient Cycling**	
				BG	

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Soil Functions		Weight	indicators	Weight		
F(ii)	**Infiltration, storage and availability of water and soil aeration**	**0.2**	**Water infiltration**	0.25		
			Kf	0.6		
			EC	0.2		
			Correlated indicators	0.2	SOC	0.5
					BD	0.5
			Water storage and availability	0.25		
			WFPS	0.25		
			MiP	0.25		
			MWD	0.25		
			EC	0.25		
			Soil Aeration	0.5		
			Map	0.5		
			TP	0.5		
F(iii)	**Sustain biological activity**	**0.2**	SOC	0.5		
			B-glucosidase	0.5		
F(iv)	**Sustain plant growth**	**0.2**	SRP	0.33		
			BD	0.33		
			Correlated indicators	0.33	SOC	0.5
					TP	0.5
F(v)	**Ability to resist to degradation**	**0.2**	MWD	0.33		
			SOC	0.33		
			Kfield	0.33		

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity; BD: bulk density; SRP: Soil resistance penetration; TP: total porosity; MiP: microporosity; MaP: Macroporosity; MWD: Mean weight diameter; BG: Beta-glucosidase activity.

5.2.5.2 Weighted addition of chemical, physical and biological components

For this strategy, the scoring of the indicators (linearly and non-linearly) were summed by categories: chemical, physical and biological, and each one of these categories was weighted by the 0,33 factor multiplying. The following Eq. 9 describes the SQI 3 and SQI 4, where *schemical* are the chemical indicators scoring and *nchemical* the numbers of chemical indicators; and the same for biological and physical components. Same as described before, SQI 3 represents the linear transformation of the dataset and SQI 4 non-linear.

$$SQI\ 3\ and\ SQI4 = 0{,}33.\frac{\sum schemical}{nchemical} + 0{,}33.\frac{\sum sbiological}{nbiological} + 0{,}33.\frac{\sum sphysical}{nphysical} \quad (9)$$

5.2.5.3 Principal component analysis

The Principal Component Analysis (PCA) was applied aiming at a minimum dataset selection. For this, the PCA was performed on 26 variables (pH, macronutrients, micronutrients, SOC, CEC, H+Al, BS, BD, SRP, TP, Mip, Map, WFPS, MWD, Kfs and enzymatic activity). As described by Lenka et al. (2022), principal components that have high eigenvalues can be considered representative to explain variability. Following the Kaiser's criteria, only the PCs with eigenvalues > 1,0 were retained, leading to six principal components and for each PC, only indicators with loading values within 10% of the highest value were retained.

In the case of more than an indicator retention, Pearson's correlation was performed to select only indicators that are not strongly related, to avoid redundance (supplementary Figure 1 – appendix C) (Chen et al., 2013; Askari and Holden., 2015). In total, nine indicators were selected: P, TN, BS, Zn, WFPS and MWD. Table 5 represents the PCA results. The SQI 5 and SQI 6 were obtained following eq. 10, where WPc is the weight referring to the relative variation of the principal component about the cumulative variation of the principal components selected and si is the indicator score, obtained linearly and non-linearly.

$$SQI\ 5\ and\ SQI\ 6 = \sum WPCi.si \quad (10)$$

Table 5. Results of Principal Components Analysis, with eigenvalues, variance and factor loadings for 26 soil quality indicators.

	Principal Components					
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Eigenvalue	12.719	3.303	2.373	1.898	1.408	1.166
Variance (%)	48.920	12.705	9.127	7.302	5.414	4.486
Cumulative % of variance	48.920	61.625	70.752	78.054	83.468	87.954
Weight	0.556	0.144	0.104	0.083	0.062	0.051
	Eigenvectors (Factor loadings)					
Soil indicators	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
pH	**-0.925**	0.163	-0.048	0.170	0.009	-0.034
P	0.100	**0.716**	0.415	0.267	0.276	-0.150
S	0.777	0.010	-0.226	0.207	0.029	-0.372
K	**0.912**	-0.211	-0.272	0.066	-0.012	0.057
Ca	-0.760	0.424	-0.270	0.352	0.054	0.040
Mg	-0.759	0.374	-0.347	0.272	-0.065	0.047
H+AL	**0.883**	0.144	-0.213	-0.152	0.053	-0.109
SOC	0.574	0.608	-0.034	-0.027	0.138	0.346
B	0.618	0.175	0.173	0.358	0.107	0.255
Cu	0.584	-0.271	0.490	0.312	-0.098	0.346
Fe	0.804	0.348	0.144	0.004	0.306	-0.100
Mn	-0.587	-0.479	0.398	0.393	-0.170	0.112
Zn	0.031	0.035	**0.783**	0.168	0.509	-0.180
CEC	0.055	**0.708**	-0.596	0.256	0.071	0.111
BS	**-0.927**	0.141	-0.063	0.295	-0.008	-0.026
TN	0.694	0.350	0.079	0.093	-0.186	**0.480**
EC	0.450	0.170	0.185	0.492	-0.181	-0.188
BD	**-0.859**	0.212	0.090	-0.296	0.223	-0.072
SRP	**0.876**	-0.264	-0.151	0.138	0.077	0.047
TP	**0.854**	-0.212	-0.089	0.300	-0.228	0.071
Mip	-0.632	-0.446	-0.204	0.228	0.325	0.284
Map	**0.906**	0.108	0.043	-0.067	-0.311	-0.138
WFPS	0.205	-0.531	-0.341	0.058	**0.647**	0.241
MWD	0.284	0.457	0.320	**-0.581**	-0.010	0.266
Kfs	0.792	-0.055	-0.116	0.281	0.131	-0.324
BG	-0.829	0.130	0.238	0.208	-0.283	0.038

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity; BD: bulk density; SRP: Soil resistance penetration; TP: total porosity; MiP: microporosity; MaP: Macroporosity; MWD: Mean weight diameter; BG: Beta-glucosidase activity.

5.2.5.4 SMAF – Soil Management Assessment Framework

The SMAF is a powerful tool related to soil quality evaluation (Karlen et al., 2019). This tool considers three steps as described by Ruiz et al. (2020): i- indicator selection, ii – indicator interpretation (non-linear curves) and iii – indicator integration. It is important highlight the SMAF was developed by United States of America (Andrews et al. 2004; Wienhold et al., 2009) and for this reason indicator selection was performed considering previous researches (Cherubin et al., 2016b; Cherubin et al.2016c; Luz et al.2019; Valani et al., 2020; Ruiz et al. 2020; Cherubin et al., 2021), once these works were developed in Brazil.

In the step 1 the indicators SOC, pH, P, K, EC, BD, WFPS and BG were selected and transforming between 0-1 through the scoring curves in the SMAF spreadsheet. As described by Luz et al. (2019), SMAF has pre-established class factors according to soil type, inherent organic matter content, texture, mineralogy, climate, slope, region, sampling time, crop, weathering class, analytical method of P content analysis and method for EC determination.

The following factors were selected respectively for each of these factor classes: organic matter - 4 (low OM), texture - 4 (clayey soils), climate factor - 1 (>550 mm of mean annual precipitation), mineralogy class - 3 (others), season code - 2 (summer), region code - 2 (humid regions), slope class - 2 (2-5% slope), P method - 5 (resin), weathering class - 2 (high weathering), crop code - 112 (soybean) and EC method - 1 (saturated paste). It is important to highlight that the determination of pH was made in $CaCl_2$, and the SMAF only considers determinations in water. The measurements were converted to pH in water using the following equation described by Cherubin et al. (2016c) and Ciprandi (1993): $pHwater = 0,890 + 0,992pHCaCl_2$ ($r^2 = 0,97$).

The SQI7 – SMAF, was obtained following the eq. 11, *schemical* are the chemical indicators scoring and *nchemical* the numbers of chemical indicators; and the same for biological and physical components.

$$SQI\ 7\ (SMAF) = 0.33.\frac{\sum schemical}{nchemical} + 0.33.\frac{\sum sbiological}{nbiological} + 0.33.\frac{\sum sphysical}{nphysical} \tag{11}$$

5.2.6 Data analyses

The dataset was initially tested for normality by the Shapiro-Wilk test ($p<0.05$) and whenever necessary data was transformed according to Box and Cox (1964). Analysis of variance (Anova) was performed ($p<0.05$) and whenever results were significant, they were compared through the Scott-Knott test ($p<0,05$). The statistical analysis was performed using the SISVAR 5.6 software (Ferreira, 2019).

Scores for soil quality indicators and the soil quality indexes in each treatment were submitted to the Scott-Knott test ($p<0.05$). The Pearson's correlation coefficient was also calculated for soil quality indicators, and between SQI indexes and crop productivity, using the package "corrplot" in the environment RStudio (1.4.1103), and the significance was analyzed by Student's t test ($p<0,05$). The PCA analysis, were performed using the packages "FactoMiner" and "factoextra" in the environment RStudio (1.4.1103).

5.3 Results

5.3.1 Slaughterhouse effluent, irrigation and nutrient supply

The physico-chemical characterization of the slaughterhouse effluent applied during the experiment is shown in Table 6. Treatment T2 – 100% TSE was the one with highest irrigation depth and TN supply for both crops and cycles (Table 7), followed by treatments T3 – 75%, T4 – 50 % and T5 – 25%, as expected. Total irrigation depth for the period from 2020 to 2022 for two cycles of black oat and two of soybean is shown in Figure 2. Nutrient supply is shown in Table 7. It is important to note that there was no nitrogen fertilization in the soybean for the treatment 0% TSE. The N supply came from the micronutrient application.

Table 4. Mean values followed by standard deviation for the physico-chemical characterization of the treated slaughterhouse effluent (TSE) and tap water (TW) between 2020 and 2022.

Parameter			**TSE**		**SW**
N-NH$_4^+$	(mg.L^{-1})	45.81	±	20.18	0.00
N-NTK	(mg.L^{-1})	60.53	±	31.60	1.68
N-NO$_3^-$	(mg.L^{-1})	1.13	±	0.71	0.80
N-NO$_2^-$	(mg.L^{-1})	0.13	±	0.14	ND
N-TN	(mg.L^{-1})	61.21	±	31.15	2.48
Ca^{+2}	(mg.L^{-1})	18.19	±	3.82	6.85
Fe^{+2}	(mg.L^{-1})	1.42	±	2.01	ND
Mg^{+2}	(mg.L^{-1})	1.83	±	0.50	0.57
Mn^{+2}	(mg.L^{-1})	0.09	±	0.04	ND
S-SO$_4$	(mg.L^{-1})	2.55	±	1.86	ND
Na$^+$	(mg.L^{-1})	34.11	±	9.58	0.80
K$^+$	(mg.L^{-1})	14.32	±	3.38	0.80
P-PO$_4^-$	(mg.L^{-1})	5.75	±	2.90	0.08
pH	-	7.87	±	0.66	6.39
EC	(dS m^{-1})	0.55	±	0.17	0.09
SAR	(mmol.L^{-1})$^{-1/2}$	2.11	±	0.68	0.26
COD	(mg.L^{-1})	461.12	±	238.76	-
TS	(mg.L^{-1})	542.08	±	303.08	159.37
TDS	(mg.L^{-1})	218	±	30.47	-

TKN: total Kjeldahl nitrogen; TN: total nitrogen; EC = electrical conductivity; SAR: sodium adsorption ratio; COD: chemical oxygen demand; TS: total solids; TDS: total dissolved solids; ND: not detectable.

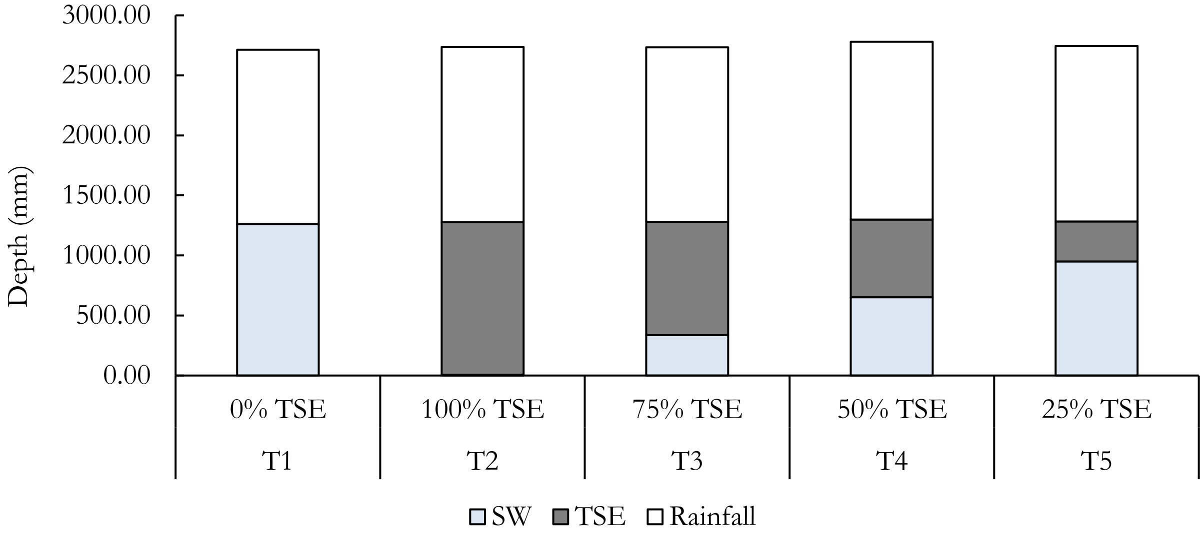

Figure 2. Depth for tap water (TW), treated slaughterhouse effluent (TSE) and rainfall, between 2020 and 2022.

Table 5. Supply of macro and micronutrients via treated slaughterhouse effluent (TSE) and conventional fertilization.

Nutrients	TN	Ca	Fe	Mg	Mn	S	Na	K	P	B	Zn
						Kg ha^{-1}					
Treatments						Black oat (2020)					
T1	87.34	28.44	0.00	2.38	0.00	0.00	3.32	33.32	0.33	0.00	0.00
T2	411.78	78.82	3.75	6.53	0.25	8.02	154.82	90.57	5.96	0.00	0.00
T3	298.71	56.60	2.70	5.35	0.18	5.76	112.11	89.38	4.37	0.00	0.00
T4	212.39	39.63	1.89	4.48	0.12	4.03	79.51	88.93	3.16	0.00	0.00
T5	123.31	22.14	1.05	3.54	0.07	2.25	45.88	87.72	1.91	0.00	0.00
						Black oat (2021)					
T1	68.90	23.84	0.00	1.99	0.00	0.00	2.78	62.78	40.28	0.00	0.00
T2	188.15	75.09	2.85	7.62	0.42	14.03	97.36	108.78	59.31	0.00	0.00
T3	144.43	62.82	2.15	6.27	0.32	10.60	74.27	97.57	54.66	0.00	0.00
T4	91.52	45.94	1.32	4.47	0.20	6.52	46.51	83.95	49.10	0.00	0.00
T5	52.58	35.99	0.70	3.34	0.10	3.43	25.86	73.98	44.93	0.00	0.00
						Soybean (2020/2021)					
T1	11.17	19.93	0.00	2.02	0.70	1.98	2.33	62.33	20.23	0.05	2.10
T2	120.64	53.59	7.78	5.07	0.87	8.31	106.46	104.37	41.36	0.05	2.10
T3	93.32	45.30	5.83	4.32	0.83	6.73	80.44	93.88	36.08	0.05	2.10
T4	71.31	40.17	4.22	3.84	0.79	5.42	59.06	85.37	31.72	0.05	2.10
T5	39.53	29.42	1.99	2.87	0.74	3.60	29.10	73.20	25.65	0.05	2.10
						Soybean (2021/2022)					
T1	9.02	14.17	0.00	1.54	0.70	1.98	1.65	31.65	20.17	0.05	2.10
T2	168.18	37.38	3.14	4.06	0.88	6.96	71.57	60.09	37.77	0.05	2.10
T3	128.43	31.67	2.36	3.44	0.83	5.72	54.10	53.00	33.37	0.05	2.10
T4	96.21	28.27	1.71	3.03	0.80	4.69	39.88	47.31	29.77	0.05	2.10
T5	50.06	20.76	0.81	2.24	0.75	3.26	19.65	39.02	24.69	0.05	2.10
						Total (2020 - 2022)					
T1	176.44	86.38	0.00	7.93	1.40	3.96	10.09	190.09	81.01	0.09	4.20
T2	888.75	244.88	17.52	23.28	2.42	37.32	430.20	363.83	144.40	0.09	4.20
T3	664.89	196.40	13.04	19.38	2.16	28.81	320.92	333.82	128.48	0.09	4.20
T4	471.44	154.00	9.14	15.82	1.91	20.66	224.97	305.56	113.75	0.09	4.20
T5	265.48	108.31	4.55	11.99	1.66	12.54	120.48	273.93	97.18	0.09	4.20

TN = total nitrogen, T1 = 0% TSE, T2 =100% TSE, T3 = 75% TSE, T4 = 50% TSE and T5 = 25% TSE.

5.3.2 Soil quality indicators

Table 8 provides information about the evaluated soil quality indicators. For chemical indicators, there was generally a decrease in depth for pH, CEC, BS and Ca. For these same indicators and additionally for Mg, the values of native vegetation (T6 - NV) were lower for treatments T1 to T5 (0 to 100% of TSE). For K, TN and H+Al, the values measured at T6 - NV were higher than at T1 - T5.

For the physical attributes, lower values for BD were found for T6 - NV in relation to the other attributes and for TP and SRP the opposite occurred. For the other physical attributes, no statistical differences were found. There were also no significant differences for the physical attributes in the 0-10, 10-20 and 20-40 cm layers, except for SRP. And for biological attributes, there was a decrease in B-glucosidase (BG) depth, with T6-NV values lower than T1 - T5. The SOC also showed a decrease in depth for T2 - 100%TSE.

Table 8. Soil quality indicators for 2022 (time 2) for the different treatments (T1 – 0%TSE, T2 – 100%TSE, T3 – 75%TSE, T4 – 50%TSE, T2 – 25%TSE and T6 – NV) and depths.

Depth (cm)	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**
			pH			
0-10	5.28 aA	5.40 aA	5.40 aA	5.38 aA	5.23 aA	4.50 aB
10-20	5.10 aA	5.15 bA	5.23 aA	5.23 aA	5.13 aA	4.33 aB
20-40	4.85 bA	5.00 bA	5.08 aA	5.10 aA	4.98 aA	4.30 aB
			P (m dm^{-3})			
0-10	14.00 aA	18.00 aA	14.25 aA	12.25 aA	15.33 aA	10.00 aA
10-20	9.50 bB	12.50 bA	11.75 bA	9.25 bB	13.67 aA	9.33 aB
20-40	7.75 bB	6.75 cB	7.33 bB	6.50 bB	9.75 bA	12.00 aA
			S (m dm^{-3})			
0-10	6.50	7.00	9.00	7.00	7.50	6.67
10-20	6.50	6.75	10.75	8.00	8.75	21.00
20-40	8.00	13.50	10.75	10.00	11.25	26.30
			K (mmol dm^{-3})			
0-10	0.68 aB	1.00 aB	0.90 aB	1.23 aB	1.10 aB	2.90 aA
10-20	0.47 aC	0.48 bC	0.73 aB	0.88 bB	0.85 aB	2.67 aA
20-40	0.28 aB	0.45 bB	0.65 aB	0.60 cB	0.73 bB	2.30 bA
			Ca (mmol dm^{-3})			
0-10	37.00 aB	40.00 aB	47.50 aA	43.75 aA	35.00 aB	19.33 aB
10-20	30.00 bC	35.50 bB	40.25 bA	33.00 bC	29.75 bC	16.33 aD
20-40	22.50 cA	28.00 cA	31.50 cA	24.00 cA	24.75 cA	15.33 aA
			Mg (mmol dm^{-3})			
0-10	13.00 aC	17.50 aB	20.50 aA	17.25 aB	11.25 aC	6.33 aD
10-20	10.75 bB	14.50 bA	16.25 bA	14.50 bA	9.50 aB	5.33 aD
20-40	7.00 bB	11.50 cA	13.25 cA	10.25 cA	7.75 aB	3.33 aC
			H+AL (mmol dm^{-3})			
0-10	33.39 aB	33.39 aB	35.71 aB	34.21 aB	44.94 aA	46.12 aA
10-20	33.38 aB	33.17 aB	36.39 aB	36.23 aB	41.48 aA	55.71 bA
20-40	34.17 aB	32.13 aB	37.37 aB	37.85 aB	39.26 aB	67.08 cA

To continue…

Continuation.

CEC (mmol dm^{-3})						
0-10	84.25 aA	92.25 aA	104.75 aA	96.75 aA	92.50 aA	85.00 aA
10-20	74.50 bA	84.00 bA	94.50 bA	84.75 bA	81.75 bA	83.00 aA
20-40	64.00 cC	73.00 cB	81.75 cA	72.75 cB	72.75 cB	84.33 aA
BS (%)						
0-10	60.27 aA	63.63 aA	65.55 bA	64.38 aA	51.30 aB	33.77 aC
10-20	55.38 bA	60.50 bA	60.30 bA	57.28 bA	49.40 aA	29.40 aB
20-40	46.83 cB	55.48 cA	55.73 aA	48.75 cB	45.88 aB	25.00 aC
TN (g kg^{-1})						
0-10	1.63 aB	1.63 aB	1.82 aB	1.71 aB	1.64 aB	2.19 aA
10-20	1.46 aB	1.39 aB	1.53 bB	1.45 aB	1.50 aB	1.86 bA
20-40	1.29 aA	1.08 bA	1.37 bA	1.21 aA	1.27 aA	1.55 bA
B (m dm^{-3})						
0-10	0.26	0.22	0.20	0.51	0.24	0.33
10-20	0.21	0.21	0.21	0.13	0.16	0.30
20-40	0.15	0.24	0.26	0.10	0.15	0.36
Cu (m dm^{-3})						
0-10	4.90	4.25	4.55	5.55	4.53	6.53
10-20	5.68	4.07	3.85	4.88	4.75	5.60
20-40	4.35	4.00	3.55	3.87	4.25	5.47
Fe (m dm^{-3})						
0-10	21.75 aC	26.75 aC	18.25 aC	22.75 aC	35.00 aB	49.67 aA
10-20	15.50 aB	16.75 bB	19.75 aB	17.25 aB	28.75 aA	29.66 bA
20-40	14.70 aB	14.00 bB	19.25 aB	13.50 aB	20.00 bB	32.00 bA
Mn (m dm^{-3})						
0-10	42.13	38.88	29.90	38.65	31.43	22.50
10-20	38.88	29.08	24.58	30.88	27.90	13.07
20-40	27.36	21.08	22.00	22.36	19.45	22.50
Zn (m dm^{-3})						
0-10	3.30 aA	2.93 aA	1.28 aA	2.10 aA	2.35 aA	1.70 aA
10-20	1.53 bA	1.30 bA	0.70 aA	0.90 bA	1.55 bA	1.03 aA
20-40	0.48 cA	0.43 cA	0.80 aA	0.38 bA	0.53 cA	1.20 aA
BD (g cm^{-3})						
0-10	1.24 aA	1.29 aA	1.23 aA	1.31 aA	1.26 aA	1.01 aB
10-20	1.28 aA	1.36 aA	1.36 aA	1.27 aA	1.36 aA	1.10 aA
20-40	1.35 aA	1.38 aA	1.33 aA	1.33 aA	1.33 aA	1.02 aB
SRP (Mpa)						
0-10	0.63 cB	0.69 bB	0.66 bB	0.58 cB	0.57 cB	1.01 bA
10-20	0.83 bB	0.98 aB	0.94 aB	0.97 aB	0.94 aB	1.10 aA
20-40	1.00 aB	0.74 bB	0.77 bB	0.83 bB	0.80 bB	1.02 aA
TP (cm^{3}.cm^{-3})						
0-10	0.57 aB	0.55 aB	0.57 aB	0.55 aB	0.56 aB	0.65 aA
10-20	0.53 aA	0.53 aA	0.53 aA	0.56 aA	0.53 aA	0.62 aA
20-40	0.56 aB	0.52 aB	0.54 aB	0.54 aB	0.54 aB	0.64 aA

To continue…

Continuation.

			MiP ($cm^3.cm^{-3}$)^{ns}			
0-10	0.40	0.41	0.39	0.44	0.39	0.35
10-20	0.41	0.41	0.41	0.44	0.42	0.36
20-40	0.44	0.45	0.40	0.43	0.41	0.37
			MaP ($cm^3.cm^{-3}$)			
0-10	0.17 aA	0.15 aA	0.19 aA	0.10 aA	0.17 aA	0.30 aA
10-20	0.11 aB	0.12 aB	0.12 aB	0.12 aB	0.12 aB	0.26 aA
20-40	0.11 aA	0.07 aA	0.13 aA	0.11 aA	0.18 aA	0.27 aA
			WFPS ^{ns}			
0-10	0.46	0.49	0.44	0.54	0.47	0.54
10-20	0.52	0.52	0.53	0.51	0.52	0.58
20-40	0.54	0.60	0.51	0.53	0.54	0.57
			MWD (mm) ^{ns}			
0-10	4.65	4.48	4.40	4.39	4.43	4.39
10-20	4.06	3.73	3.89	4.12	4.26	3.92
20-40	3.46	2.83	3.35	3.25	3.74	3.95
			BG (mg .Kg^{-1} .h^{-1})			
0-10	64.50 aA	70.50 aA	61.50 aA	65.75 aA	46.00 aB	21.33 aC
10-20	46.00 bA	38.50 bA	41.50 bA	39.50 bA	39.25 bA	14.33 aB
20-40	22.25 cA	26.00 bA	23.75 cA	26.00 bA	23.50 bA	10.33 aA
			SOC (g kg^{-1})			
0-10	12.85 aA	13.76 aA	12.92 aA	13.18 aA	12.94 aA	13.84 aA
10-20	12.07 aA	11.76 bA	12.33 aA	12.20 aA	12.55 aA	12.70 aA
20-40	11.01 aA	10.43 bA	11.76 aA	10.86 aA	11.43 aA	12.28 aA

T1 = 0% TSE, T2 = 100% TSE, T3 = 75% TSE, T4 = 50% TSE , T5 = 25% TSE, T6 – NV; P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity; BD: bulk density; SRP: Soil resistance penetration; TP: total porosity; MiP: microporosity; MaP: Macroporosity; MWD: Mean weight diameter; BG: Beta-glucosidase activity. Same small letters in columns and capital letters in rows do not differ from each other by the Scott-Knott test ($p<0.05$).

5.3.3 Soil quality indexes strategies

As described in the item 2.5 of the methodology, the indicators were scored based on linear and non-linear transformations. This resulted in different results when comparing the seven index strategies. Figure 3a, compares the different index approaches (SQI1 to SQI7) for the treatments T1 to T6 and the Figure 3b, brings the differences between the treatments T1 to T6 inside each index approach.

According to Figure 3, SQI1, SQI2, SQI3 and SQI4 did not present differences between the treatments T1 to T6. For SQI5, treatment T6 showed the smallest score in comparison to T1-

T5; for SQI6, T1, T2 and T5 presented higher scores in comparison to T3, T4 and T6 and, for SQI7 treatment T6 was superior to T1-T5. Analyzing Figure 2, for treatments T1, T3, T4 and T5, the indexes SQ1, SQI3 and SQI5 did not present differences. Except for T6, for all treatments, the smallest scores were verified for SQI2 and SQI4. For T6, SQI2, SQI4 and SQI6, didn't show differences between themselves, same for SQI1 and SQI7, and the smallest SQI was verified for SQI5.

Analyzing the sensitivity of the seven soil quality index strategies (Figure 4), SQI5 and SQI6, strategies based on PCA, showed the greatest sensitivity in comparison to the others. These two strategies are the most complex of the seven SQI evaluated, followed by SQI7 – SMAF. This indicates the possibility of reducing the initial dataset into a small group of indicators, without losing sensitivity. SQI7-SMAF only considers a maximum of 12 indicators and in this present study, 8 indicators were used, as described in session 2.5.

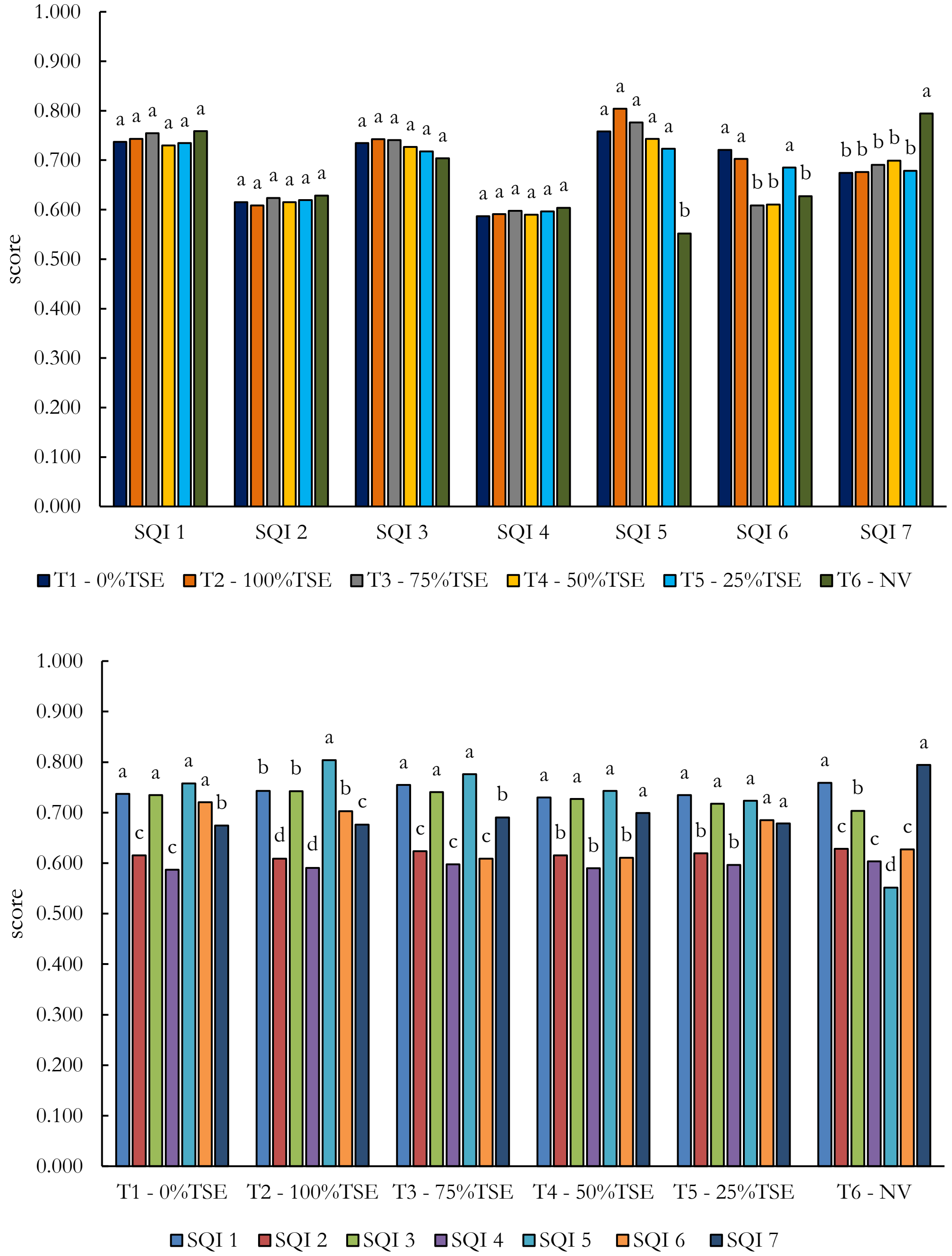

Figure 3. a) Treatments comparison inside each Soil quality index (SQI) strategies and b) Soil quality Indexes (SQI) comparisons for treatments T1 to T6, where T1 – 0%TSE, T2 – 100%TSE, T3 – 75%TSE, T4 – 50%TSE, T5 – 25%TSE and T6 – NV. SQI1 – Soil quality index based on soil functions with linear transformation; SQI2 - Soil quality index based on soil functions with non-linear transformation; SQI3 – Soil quality index based on weight additive with linear transformation; SQI4 - Soil quality index based on soil functions with non-linear transformation; SQI5 – Soil quality index based on PCA analysis with linear transformation; SQI6 - Soil quality index based on PCA analysis with non-linear transformation and SQI7 – Soil quality index based on SMAF tool. Means followed by the same letters do not differ according to the Scott-Knott test ($p<0.05$).

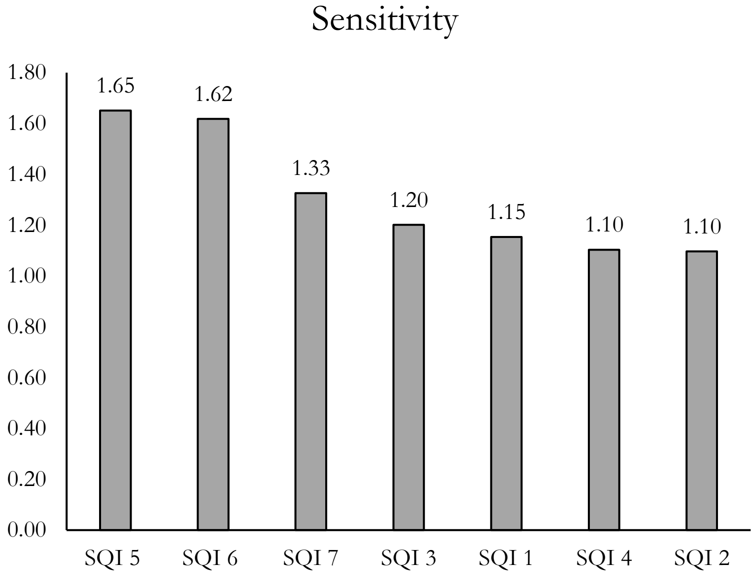

Figure 4. Sensitivity values of SQI strategies for evaluate the soil quality provided by irrigation with different doses of treated slaughterhouse effluent (TSE) and for a native vegetation. SQI1 – Soil quality index based on soil functions with linear transformation; SQI2 - Soil quality index based on soil functions with non-linear transformation; SQI3 – Soil quality index based on weight additive with linear transformation; SQI4 - Soil quality index based on soil functions with non-linear transformation; SQI5 – Soil quality index based on PCA analysis with linear transformation; SQI6 - Soil quality index based on PCA analysis with non-linear transformation and SQI7 – Soil quality index based on SMAF tool.

5.3.4 Correlation between soil quality indexes, carbon stocks and crop productivity

Although no statistical differences were detected for carbon stocks between different slaughterhouse effluent doses (T1 to T5) and native vegetation area (T6) (Figure 5a), it is important to assess their impact on crop productivity and soil quality indexes.

There was no correlation between black oat, soybean and cumulative yields with soil quality indexes and carbon stocks (Figure 5b). Carbon stocks also only showed significant positive correlations with the SQI1, SQI2, SQI3 and SQI4 indexes, indicating that the increase in these carbon stocks led to an increase in soil quality indexes. In general, the soil quality indexes were all positively correlated with each other, except for SQI 7 (based on the SMAF tool). The SQI7 only presented significative positive Pearson's correlations with SQI1 and SQI3.

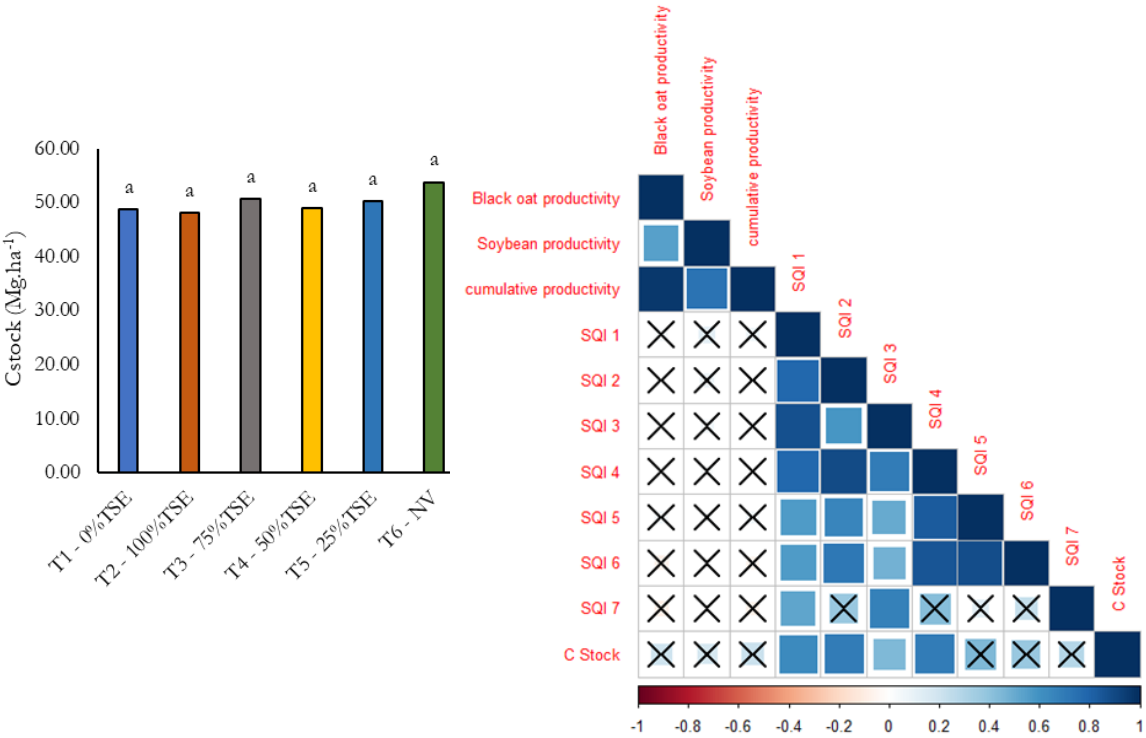

Figure 5. a) Carbon stocks for treatments T1 to T6, where T1 – 0% TSE, T2 – 100% TSE, T3 – 75% TSE, T4 – 50% TSE, T5 – 25% TSE and T6 – NV (Means followed by the same letters do not differ according to the Scott-Knott test ($p<0.05$)). b) Person's correlation between SQI1 – Soil quality index based on soil functions with linear transformation; SQI2 - Soil quality index based on soil functions with non-linear transformation; SQI3 – Soil quality index based on weight additive with linear transformation; SQI4 - Soil quality index based on soil functions with non-linear transformation; SQI5 – Soil quality index based on PCA analysis with linear transformation; SQI6 - Soil quality index based on PCA analysis with non-linear transformation, SQI7 – Soil quality index based on SMAF tool, Cstock – Carbon stock, black-oat productivity, soybean-productivity and cumulative productivity. Values containing an "x" did not show significant correlations ($p<0.05$).

5.4 Discussion

5.4.1 Slaughterhouse effluent, irrigation and nutrient supply

As exposed by table 6 and according to resolution number 420 from the Brazilian National Environment Council (Conama, 2011), the effluent would not be suitable for disposal in water bodies as the ammoniacal nitrogen content is over the critical limit (20 mg.L^{-1}). However, according to resolution number 503 (Conama, 2021) regarding the reuse of agro-industrial effluents, the effluent is classified as suitable for agricultural reuse because the resolution mentions that parameters of agronomic interest are not required to follow resolution 420 from 2011.

According to Raij et al. (1996), 20 kg ha^{-1} of N is recommended for black oat during sowing and after each cut. In the first cycle of black oat (2020) 80 kg ha^{-1} by means of urea was applied in T1 – 0%TSE, while 60 kg ha^{-1} was applied in the second cycle. It is possible to verify that during the first crop cycle for treatments T2 to T5, nutrient supply was higher than the recommended for fertilization (Table 6). Similarly, nutrient supply in treatments T2 to T4 were higher than the recommended in the second cycle (2021), while it was lower for T5 (52.58 kg ha^{-1}). In relation to soybean, Dos Santos Cordeiro and Echer (2019) suggest a supply of 50 kg ha^{-1} of N combined with biological nitrogen fixation to increase soil N and crop yield. In this view, treatment T5 is within the recommended range.

5.4.2 Soil quality indicators

Analyzing the chemical indicators of soil quality, it is possible to observe that for phosphorus and potassium, these values are below the recommended for Brazilian soils and cultures in question (Raij et al., 1996). On the other hand, the contents of Ca, Mg, and micronutrients are high considering what is recommended by Raij et al. (1997). Matheyarasu et al. (2017) and Seshadri et al. (2014) also found that adding TSE can increase B, Zn, Mn, Cu, and Fe levels. These trends are similar to what was presented by Menegassi et al. (2020) due to the same experimental conditions and study location. It is also important to emphasize that for Ca and Mg, treatments T1 and T6 did not receive a constant supply of these nutrients and that T5 received the lowest doses compared to the others supplied by T2, T3, and T4. Therefore, statistical differences in all layers for these two nutrients were verified.

As expected, in most of the chemical indicators, T6 showed the lowest values for the indicators (except for TN and K) once did not receive a constant supply of nutrients by TSE. Nitrogen is very dynamic in soils, and its loss via NO, N_2O and N_2 can occur soon after fertilizer application, about one day (Dalal et al., 2003). The application of TSE as a source of N and water causes a permanently humid environment, which can lead to the denitrification process (Matheyarasu et al., 2016; Wrage-Mönnig et al., 2018; Wrage et al., 2001).

For physical indicators, aligned to Coelho et al. (2020), the irrigation with TSE did not change physical indicators, especially because this type of change takes long periods. Except for SRP, T6-VN was superior to the treatments T1 – T5 due to SOM, although statistically the values for this attribute were the same for all treatments. Li and Shao (2006), attested to the recovery of soil structure through the regeneration of native forests, which indicates the inherent physical quality of these areas not disturbed by anthropic actions. About SRP, there is a close connection between this indicator and soil moisture content (Moraes, 2013; Fernandes et al., 2020), where the increase in soil moisture led to a decrease in SRP. As the experimental field was constantly irrigated, probably this is the reason SRP shows the biggest values to T6-NV.

Regarding biological indicators, SOC didn't show any statistical difference for treatments T1-T6 and for beta-glucosidase activity, the values found for T6 – NV were much smaller than the experimental field. The beta-glucosidase acts in the final step of cellulose decomposition (Tabatai,1994), It's connected with the C-cycle and it's a good indicator to detect land use changes, soil management and the changes related to SOM (Adetunji et al., 2017; Gunal et al., 2018). Soil beta-glucosidase is very dependent on soil moisture and soil pH, and the decrease of soil moisture causes the activity decrease of this enzyme (Sardans and Penuela, 2005). For this reason, T6 – NV had the lowest values in comparison to the irrigated treatments. The collection samples procedure can also be influenced by these results, once this collection was made on a drought summer day.

According to Mendes (2015), the beta-glucosidase activity values in Brazilian soils considered low, medium and high are respectively <60, 61-140 and >140 mg $kg^{-1}.h^{-1}$. In comparison with the results of this study, in all treatments the values were low. There is no consensus on the impact of effluent application on soil enzymes. This is because few studies have evaluated these impacts. Subrahmanyam et al. (2016) evaluating the application of industrial effluents on the soil, detected a decrease in beta-glucosidase activity with the increase in applied doses. In contrast, Yan and Pan (2010), detected that the application of wastewater in short periods of irrigation increased the enzymatic activity of beta-glucosidase. The justification, in this case, is linked to the supply of organic matter in readily decomposable forms, increasing microbial activity.

5.4.3 Soil quality indexes

As explained in topic 5.3.3, only the SQI5, SQI6 and SQI7 indexes detected statistical differences between treatments T1-T6. As well as the one found by Barbosa et al. (2018), the different doses of treated slaughterhouse effluent did not impact the evaluated SQI1, SQI2, SQI3 and SQI4. For the approaches involving PCA, T6 was inferior to treatments irrigated with TSE in SQI5 and in SQI6 similar to T3 and T4. The reasons for this are linked to the BS indicator having received the highest weight in the composition of the index according to the PCA (Table 5) and to T6 having presented the lowest values compared to T1-T5 (Table 8). For SQI7 the opposite was verified, since T6 was superior to the other treatments. This is linked to the fact that T6 received the highest scores in the physical and biological components, since the highest scores for BD and SOC were obtained in T6 (supplementary figure 2 and supplementary table 2 – appendix C).

Compared to strategies that used the entire dataset (SQI1, SQI2, SQI3 and SQI4), the highest sensitivities (figure 4) were found for strategies that considered a minimal dataset (SQI5, SQI6 and SQI7). This indicates the possibility of using a minimum set to assess soil quality in sites irrigated with agroindustrial effluents, such as the TSE. However, the limitation of the strategies involving PCA in this case is the fact that no biological indicator was selected through the PC selections following Kaiser's criteria and loading values. Thus, the SQI7 would be the most suitable for assessing the SQ in the present study. Although SMAF has been developed for North American soils (Andrews et al., 2004), its applicability in tropical soils has been proven (Ruiz et al., 2020; Cherubin et al., 2017; Luz et al., 2019).

Although the study of soil quality through the analysis of soil functions is widely used and recommended (Fernandes et al., 2011; Lima et al., 2013; Cherubin et al., 2016; Barbosa et al., 2018; Silva-Olaya et al., 2022), for the present study, there was no sensitivity of this approach for differentiating the treatments applied (SQI1 and SQI2). The same was verified when the scoring of the indicators (linearly and non-linearly) was summed by categories: chemical, physical and biological, and weighted by the 0.33 factor multiplying (SQI 3 and SQI4).

When comparing linear and non-linear data transformation strategies, for treatments from T1 to T5, the pattern was the same. Linear approaches, even with different integrations for SQI composition, did not show statistical differences, and the same for non-linear approaches. The exception only occurred for SQI7, which despite presenting the use of non-linear curves by the SMAF tool, was similar to the linear approaches for T4 and T5. For T6, the SQI with a non-linear approach (except SQI7) was similar, but for linear scoring, this resulted in different scores.

The great advantage of using linear data transformation techniques is the associated mathematical simplicity and the lack of prior knowledge about the evaluated area (Yu et al., 2018a; Zhou et al., 2020). However, studies have shown that non-linear scoring methods are better when applied to soil functions (Andrews et al., 2002; Askari and Holden, 2015; Yu et al., 2018a; Nabiollahi, 2018), are not restricted by area (Zhou et al., 2020), but require more knowledge associated with the reference values behind the nonlinear curves used for scoring (Andrews et al., 2002b; Raiesi et al., 2017; Yu et al., 2018).

In agreement with the cited works, the selection of a minimum set of indicators, with non-linear transformation of the data seems adequate to the studies involving TSE application. The linear transformation of the data seems to be suitable for a local scale, while the non-linear transformation, which relies on values from the literature, can be useful in studies to compare the regional scale.

5.4.4 Correlation between soil quality indexes, carbon stocks and crop productivity

As explained in topic 5.3.4, the only index that did not show significant positive correlations with SQI2, SQI4, SQI5 and SQI6 was SQI7. Furthermore, although the SQI7 is based on the SMAF tool, with non-linear transformations of the indicators, positive correlations occurred with the indexes that presented linear transformations of the indicators (SQI1 and SQI3). SQI1 is based on soil functions and SQI3 on weight additive technique, and both measured the soil quality using the entire dataset. In general, the indexes that received the same type of data transformation (linear and non-linear) and the same type of indexing (TDS or MDS), were positively and highly correlated with each other. This was also identified in the work of Nabiollahi et al. (2019) and Zhou et al. (2020).

As reported by Cherubin et al. (2016), the sensitivity of the indexes declined with the transition from MDS to TDS. Other works also report that the use of an MDS can be effective in assessing soil health, but without consensus between linear and non-linear data transformation (Askari and Holden, 2015; Zhou et al., 2020; Mahajan et al., 2021). Specifically for works that evaluated the application of effluent on soil quality, which are few in the literature, the trend found was the same (Rezapour et al., 2021).

According to highly cited papers in the literature (Doran and Parkin, 1994; Gregorich et al., 1994; Reeves, 1997), soil organic carbon plays a central role in soil quality and soil health. This is due SOC is highly connected with all of the other indicators of soil quality: SOC is very important for microbial processes, nutrient availability, and physical structure/stability (Reeves, 1997; Hoffland et al., 2020). For this reason, an MDS must contain this indicator. However, analyzing Pearson's correlation between C stocks and the SQI indexes and crop productivity, the positive correlations only occurred for indexes that considered the TDS (SQI1, SQI2, SQI3 and SQI4). This may have occurred because in SQI5 and SQI6, through PCA, the SOC indicator was not selected, and it directly influences the calculation of carbon stocks.

Although, as previously discussed, TSE provides nutrients and organic matter, which directly affect soil health and crop productivity, there were no correlations between any of these indexes and crop productivity. This result goes against that identified by Rezapour et al. (2021) and Lenka et al. (2022), but according to what was found by Armenise et al., (2013) and Amorim et al. (2020). Due to the experimental conditions outside the protected environment, climatic influences as well as other agents such as diseases and pests may have affected crop productivity beyond the TSE application.

Analyzing all the conditions previously discussed, in this way, the SQI7 - SMAF, configures the best index to evaluate the effects of the application of different doses of TSE on soil quality. This is because, despite being the third most sensitive index, it included SOC in its MDS, could distinguish T6 - NV from T1 to T5 (experimental area) and is already well established for tropical soils (although it did not show correlations with Cstocks and crop productivity).

5.5 Conclusions

Regarding soil quality indicators, in general, the different TSE doses (T1 - T5) did not result in statistical differences for chemical, physical and biological indicators. Also, for some chemical indicators (pH, CEC, BS and Ca), there was a decrease with depth and in general, T6 - NV presented the lowest averages compared to the treatments that received TSE (Except for K).

For the physical indicators, the opposite was verified, T6-NV presented the best results in opposition to T1 - T5, except for SRP. The different TSE doses also did not lead to different results for the physical indicators. About SRP, there is a close connection between this indicator and soil moisture content, where an increase in soil moisture leads to a decrease in SRP. As the experimental field was constantly irrigated, this is probably the reason SRP shows the biggest values for T6-NV. Regarding biological indicators, SOC didn’t show any statistical difference for treatments T1-T6 and for beta-glucosidase activity, the values found for T6 – NV were much smaller than the experimental field.

The only soil quality indexes that detected statistical differences between treatments were SQI5, SQI6 and SQI7, based on an MDS. These differences were mainly detected between T6 - NV and the other treatments, as expected, but not between different TSE doses (except for SQI6). The highest sensitivities were also detected for these indexes.

In general, the indexes that received the same type of data transformation (linear and non-linear) and the same type of indexing (TDS or MDS) were positively and highly correlated with each other. However, there was no correlation between indexes and Cstocks with crop productivity. No differences were identified between the Cstocks for treatments T1 - T6 either.

Therefore, although the different doses of TSE have not caused an increase in soil quality indexes, they do not pose any risks to its reuse either. The functioning capacities of the area that received irrigation with native vegetation were very close to each other. This is positive from an environmental point of view since the irrigation of the cultures in question can be performed with the TSE without losing soil quality and maintaining the preservation of water resources.

In addition, based on the results presented, it is recommended that the impact of agro-industrial effluents on QS be studied using an MDS that includes at least physical, chemical and biological indicators. As no biological indicator was selected using the PCA technique, the best strategy to assess QS was the one promoted by the SQI7 - SMAF index, which considers chemical, physical and biological indicators

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6. FINAL REMARKS

In this study, we were able to demonstrate that the use of treated slaughterhouse effluent can be a viable measure for the reuse of water, rich in nutrients such as N, P, and K, as well as organic matter. Besides being an environmentally sound measure, preventing water pollution, it can contribute to water conservation and partial replacement of nutrients in crops of agricultural interest. Aligned with the 17 Sustainable Development Goals (SDGs), the agricultural reuse of this type of effluent can contribute to SDG 2 - Zero Hunger; 6 - Clean Water and Sanitation; 11 - Sustainable Cities and Communities; and 12 - Responsible Consumption and Production. Coupled with soil health, this type of effluent can have a positive impact on soil quality restoration, primarily through nutrient availability.

According to Chapter 1, it became evident that the reuse of treated slaughterhouse effluent (TSE) is not very frequently studied, mainly due to the challenges associated with the experimental conditions of this type of research. However, among the studies that aimed to assess the impacts of TSE on soil quality (SQ), chemical indicators were the most frequently evaluated, while physical indicators, followed by biological ones, were less investigated. Since SQ encompasses all three spheres of indicators (chemical, physical, and biological), it is suggested that these studies increasingly incorporate indicators from all three fronts.

Regarding Chapter 2, the assessment of physical indicators (BD, SRP, WFPS, Map, TP, SSI, MWD, and Kf) indicated that the different doses of treated slaughterhouse effluent (TSE) did not directly impact the improvement of these indicators. However, the 75% and 25% TSE doses positively affected the physical quality index over the two experimental years (2020 - 2022). From the perspective of soil chemical quality (Chapter 3) and more specifically soil functions, the application of TSE in various doses generally led to an increase in macro and micronutrients, with negative effects only on potassium levels. In line with Chapter 2, the dose that had the most positive impact on chemical soil quality indicators was 75% TSE, while the lower doses (0% and 25% TSE) resulted in the lowest SQCI, in conjunction with the native vegetation area.

In Chapter 4, the overall soil quality/health was assessed, and it was explicitly stated that different doses of treated slaughterhouse effluent (TSE) did not result in distinct Soil Quality Indices (SQI), although differences were observed when compared to the native vegetation area. This chapter also highlighted that the best strategies for evaluating SQ under experimental conditions like those in this study are based on a Minimum Dataset (MDS), with the SMAF tool standing out. In addition to facilitating the nonlinear transformation of indicators, SMAF is adapted to Brazilian soils as per the referenced research and relies on a small set of indicators. This can make SQ assessment more objective and cost-effective.

Overall, there is a noticeable lack of studies aimed at evaluating TSE's impact on physical and biological indicators and a scarcity of studies assessing soil quality indices in TSE-irrigated systems. This study is expected to inspire future research to conduct more measures like this one, directly aligned with the concept of a circular economy. We are in a transitional agricultural model where regenerating systems with maximum resource reuse are being sought. However, for these decision-making processes, research related to the topic is fundamental and guiding in this context.

APPENDICES

Appendix A

Supplementary table 1. Database considered for this bibliometric review (n=29).

Authors	**Title**
Wells and Whitton (1970)	The influence of meatworks effluents on soil and plant composition
Tate (1973)	Respiratory activity of soils irrigated by water and by meatworks effluent a note
Russel (1982)	Interaction of slaughterhouse effluent protein with three New Zealand soils
Bole and Gould (1985)	Irrigation of forages with rendering plant wastewater forage yield and nitrogen dynamics
Chuarchman and Tate (1986)	Effect of slaughterhouse effluent and water irrigation upon aggregation in seasonally dry New Zealand soil under pasture
Russell, Cooper and Lindsey (1993)	Soil denitrification rates at wastewater irrigation sites receiving primary treated and anaerobically treated meat processing effluent
Balks, Mclay and Harfoot (1997)	Determination of the progression in soil microbial response and changes in soil permeability following application of meat processing effluent to soil
Magesan et al. (1999)	Preferential flow and water quality in two New Zealand soils previously irrigated with wastewater
Guo and Sims (2000)	Effect of meatworks effluent irrigation on soil tree biomass production and nutrient uptake in eucalyptus globulus seedlings in growth cabinets
Guo and Sims (2003)	Soil response to eucalypt tree planting and meatworks effluent irrigation in a short rotation forest regime in New Zealand
Luo, Lindsey and Xue (2004)	Irrigation of meat processing wastewater onto land
Adesemoye, Opere and Makinde (2006)	Microbial content of abattoir wastewater and its contaminated soil in Lagos Nigeria
Bhandral et al. (2007)	Nitrogen transformation and nitrous oxide emissions from various types of farm effluents
Osemwota (2010)	Effect of abattoir effluent on the physical and chemical properties of soils
Da Silva Neto et al. (2013)	Chemical properties in entisol under pasture grass marandu fertilizer of liquid waste of bovine slaughter
Liu and Haynes (2013)	Effect of disposal of effluent and paunch from a meat processing factory on soil chemical and microbial properties
Arku and Musa (2014)	The effect of moringa treated wastewater on drip irrigated sandy loam soil
Seshadri et al. (2014)	Effect of industrial waste products on phosphorus mobilisation and biomass production in abattoir wastewater irrigated soil
Abegunrin et al. (2016)	Impact of wastewater irrigation on soil physicochemical properties growth and water use pattern of two indigenous vegetables in southwest Nigeria
Matheyarasu, Bolan and Naidu (2016)	Abattoir wastewater irrigation increases the availability of nutrients and influences on plant growth and development
Matheyarasu et al. (2016)	Assessment of nitrogen losses through nitrous oxide from abattoir wastewater irrigated soils
De oliveira et al. (2017a)	Percolate quality in soil cultivated with application of wastewater from swine slaughterhouse and dairy products
De oliveira et al. (2017b)	Performance of tifton 85 grass under fertirrigation with slaughterhouse wastewater

Luchese et al. (2017)	Ambiental impacts caused by the application of poultry slaughterhouse wastewater on soils
Matheyarasu et al. (2017)	Nutrient budgeting as an approach to assess and manage the impacts of long term irrigation using abattoir wastewater
Shilpi et al. (2018)	Comparative values of various wastewater streams as a soil nutrient source
Alabi et al. (2019)	Effects of different land uses on soil physical and chemical properties in Odeda Iga Ogun state Nigeria
Araújo et al. (2019)	Reforested soil under drip irrigation with treated wastewater from poultry slaughterhouse
Menegassi et al. (2020)	Reuse in the agroindustrial irrigation with treated slaughterhouse effluent in grass

Supplementary Table 2 - Experimental conditions of the 29 works reviewed

Authors	**Publication title**	**Experimental condition**
Wells and Whitton (1970)	The influence of meatworks effluents on soil and plant composition	Field experiment
Tate (1973)	Respiratory activity of soils irrigated by water and by meatworks effluent a note	Field experiment
Russel (1982)	Interaction of slaughterhouse effluent protein with three new zealand soils	not mentioned
Bole and Gould (1985)	Irrigation of forages with rendering plant wastewater forage yield and nitrogen dynamics	Field experiment
Chuarchman and Tate (1986)	Effect of slaughterhouse effluent and water irrigation upon aggregation in seasonally dry new zealand soil under pasture	Field experiment
Russell, Cooper and Lindsey (1993)	Soil denitrification rates at wastewater irrigation sites receiving primarytreated and anaerobically treated meatprocessing effluent	Field experiment
Balks, Mclay and Harfoot (1997)	Determination of the progression in soil microbial response and changes in soil permeability following application of meat processing effluent to soil	not mentioned
Magesan et al. (1999)	Preferential flow and water quality in two new zealand soils previously irrigated with wastewater	Field experiment
Guo and Sims (2000)	Effect of meatworks effluent irrigation on soil tree biomass production and nutrient uptake in eucalyptus globulus seedlings in growth cabinets	Pot experiment
Guo and Sims (2003)	Soil response to eucalypt tree planting and meatworks effluent irrigation in a short rotation forest regime in new zealand	Field experiment
Luo, Lindsey and Xue (2004)	Irrigation of meat processing wastewater onto land	Field experiment
Adesemoye, Opere and Makinde (2006)	Microbial content of abattoir wastewater and its contaminated soil in lagos nigeria	not mentioned
Bhandral et al. (2007)	Nitrogen transformation and nitrous oxide emissions from various types of farm effluents	Field experiment
Osemwota (2010)	Effect of abattoir effluent on the physical and chemical properties of soils	not mentioned
Da Silva Neto et al. (2013)	Chemical properties in entisol under pasture grass marandu fertilizer of liquid waste of bovine slaughter	Field experiment
Liu and Haynes (2013)	Effect of disposal of effluent and paunch from a meat processing factory on soil chemical and microbial properties	Field experiment
Arku and Musa (2014)	The effect of moringatreated wastewater on dripirrigated sandy loam soil	Field experiment
Seshadri et al. (2014)	Effect of industrial waste products on phosphorus mobilisation and biomass production in abattoir wastewater irrigated soil	Pot experiment
Abegunrin et al. (2016)	Impact of wastewater irrigation on soil physicochemical properties growth and water use pattern of two indigenous vegetables in southwest nigeria	Pot experiment

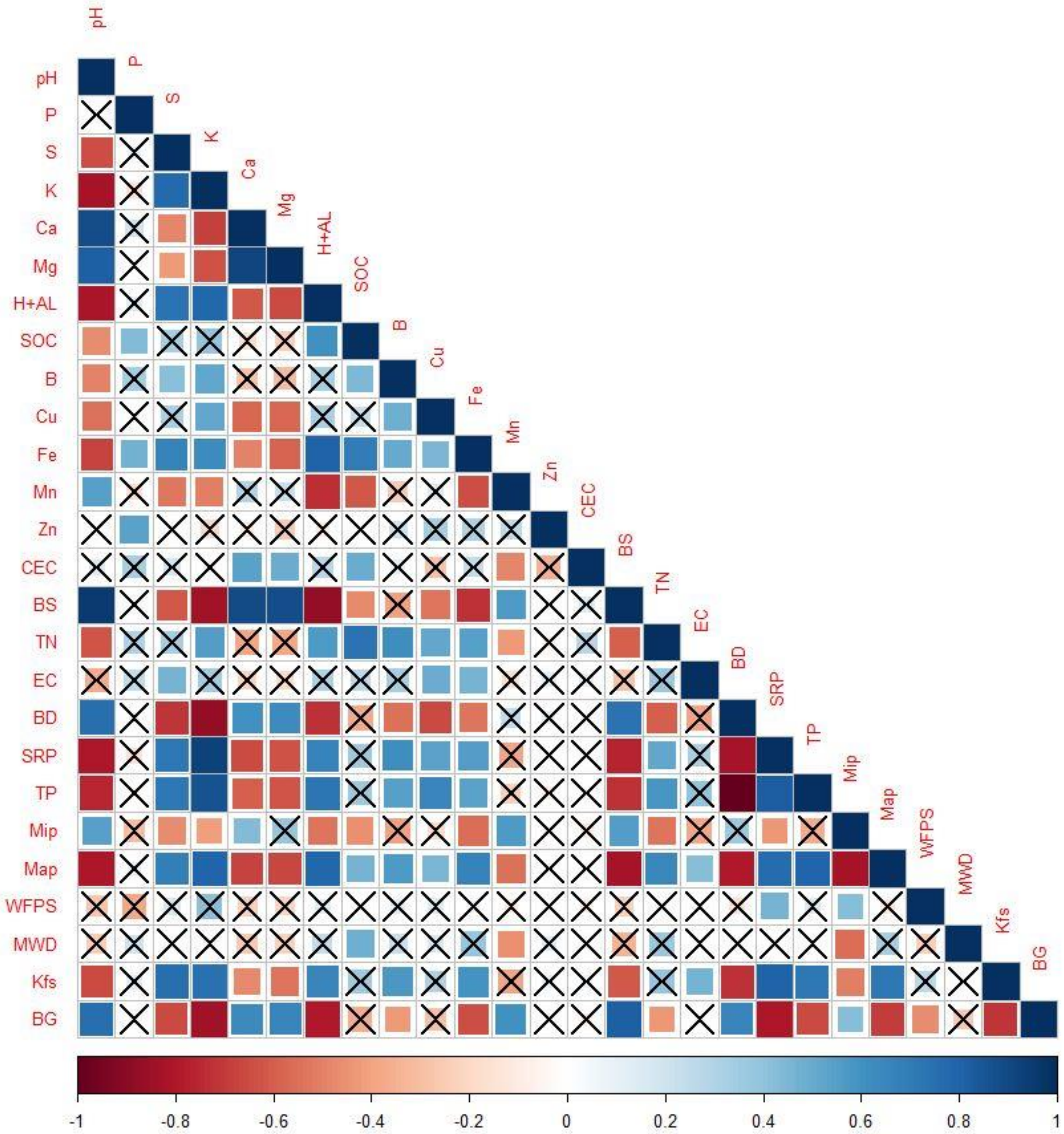
Matheyarasu, Bolan and Naidu (2016)	Abattoir wastewater irrigation increases the availability of nutrients and influences on plant growth and development	Pot experiment
Matheyarasu et al. (2016)	Assessment of nitrogen losses through nitrous oxide from abattoir wastewater irrigated soils	Pot experiment
De oliveira et al. (2017)	Percolate quality in soil cultivated with application of wastewater from swine slaughterhouse and dairy products	Soil column
De oliveira et al. (2017)	Performance of tifton 85 grass under fertirrigation with slaughterhouse wastewater	Soil column
Luchese et al. (2017)	Ambiental impacts caused by the application of poultry slaughterhouse wastewater on soils	Soil column
Matheyarasu et al. (2017)	Nutrient budgeting as an approach to assess and manage the impacts of long term irrigation using abattoir wastewater	Field experiment
Shilpi et al. (2018)	Comparative values of various wastewater streams as a soil nutrient source	Pot experiment
Alabi et al. (2019)	Effects of different land uses on soil physical and chemical properties in odeda lga ogun state nigeria	not mentioned
Araújo et al. (2019)	Reforested soil under drip irrigation with treated wastewater from poultry slaughterhouse solo reflorestado sob irrigação por gotejamento com efluentes tratados de abatedouro de aves	Field experiment
Menegassi et al. (2020)	Reuse in the agroindustrial irrigation with treated slaughterhouse effluent in grass	Field experiment

Appendix B

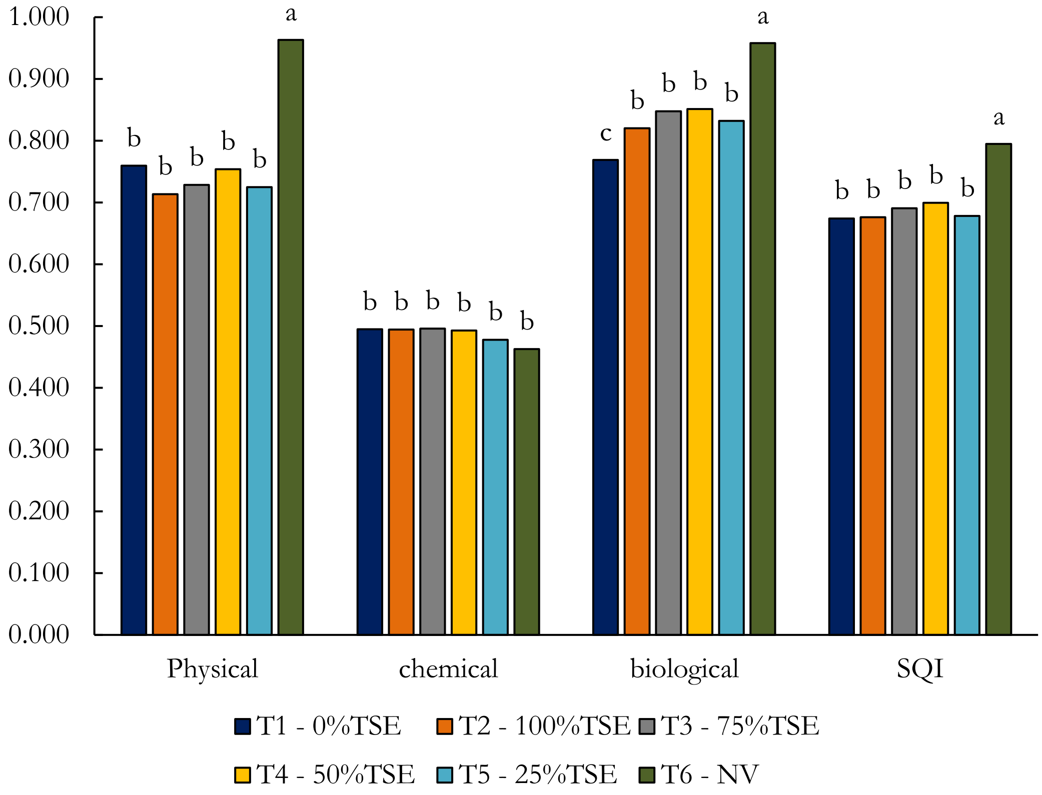
Supplementary Table 1. Soil functions, soil indicators, weights related, and soil physical quality index.

Soil Functions		Weight	Indicators	Weight	SPQI
F (i)	support roots growth	0,25	BD	0,125	
			PR	0,125	
F (ii)	supply water for plants	0,25	Kfield	0,125	$\sum Sfi.weight$
			WFPS	0,125	
F (iii)	soil aeration	0,25	Map	0,125	
			TP	0,125	
F (iv)	ability to resist to soil degratation	0,25	MWD	0,125	
			SSI	0,125	

Appendix C



Suplemmentary figure 1. Pearson correlation ($p<0.05$) between the evaluated soil quality indicators. P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity; BD: bulk density; SRP: Soil resistance penetration; TP: total porosity; MiP: microporosity; MaP: Macroporosity; MWD: Mean weight diameter; BG: Beta-glucosidase activity.



Suplemmentary figure 2. Treatments comparison for each component (physical, chemical and biological) for the SQI7 – SMAF.

Suplemmentray table 1. Indicator thresholds and scoring curves.

indicator	Unit	Lower Threshold	Lower Baseline	Upper Threshold	Upper Baseline	Optimum point	Scoring Curve	Reference
pH	unitless	4	4,5	8	7,5	5,5	Optimum	Raij et al. 1997
P	m dm-^{3}	2	8	16			More is better	Raij et al. 1997
S	m dm-^{3}	2,5	5	10			More is better	Raij et al. 1997
K	mmolc.dm-^{3}	0,4	0,8	1,6			More is better	Raij et al. 1997
Ca	mmolc.dm-^{3}	0	20	40			More is better	Silva Olaya 2022
Mg	mmolc.dm-^{3}	1	4	7			More is better	Silva Olaya 2022
H+AL	mmolc.dm-^{3}	40	80	100			Less is better	Raij et al. 1997
B	m dm-^{3}	0,1	0,3	0,6			More is better	Raij et al. 1997
Cu	m dm-^{3}	0	0,75	2,7			More is better	Silva Olaya 2022
Fe	m dm-^{3}	0	17	63			More is better	Silva Olaya 2022
Mn	m dm-^{3}	0	5	18			More is better	Silva Olaya 2022
Zn	m dm-^{3}	0	1	3,5			More is better	Silva Olaya 2022
CEC	mmolc.dm-^{3}	50	75	150			More is better	CQFS 2004
BS	%	20	40	80			More is better	Raij et al. 1997
EC	dS m^{-1}	0,7	3				Less is better	Ayers and Westcot (1999)
BD*	g cm-^{3}	0,75	1,25	1,75			Less is better	Lima et al 2013
SRP	Mpa	2	3	5			Less is better	Arshad et al., 1996
TP	m^{3} m^{-3}	0,31	0,45	0,63			More is better	Silva Olaya 2022
Mip	m^{3} m^{-3}	0	0,3	0,6			More is better	Silva Olaya 2022
Map	m^{3} m^{-3}	0,05	0,075	0,15			More is better	Cherubin et al., 2016
WFPS	unitless	0,15	0,3	0,9	0,8	0,6	Optimum	Cherubin et al., 2016
MWD	mm	0,5	3	5			More is better	Silva Olaya 2022
Kfs	cm.h-^{1}	2	7,5	15			More is better	Lopes et al., 2013
BG	mg kg-^{1}.h-^{1}	60	90	120			More is better	Cherubin et al., 2016
SOC	g kg-^{1}	2	17,5	25			More is better	Cherubin et al., 2016
TN	g kg-^{1}	1	1,75	2,5			More is better	Cherubin et al., 2016

*clay soils

P: phosphorus; S: sulfur; K: potassium; Ca: calcium; Mg: magnesium; H+Al: potential acidity; SB: sum of basis; CEC: cation exchange capacity; OM: organic matter; SOC: soil organic carbon; TN: total nitrogen; B: boron; Cu: copper; Fe: iron; Mn: manganese; Zn: zinc; BS: base saturation; EC: electrical conductivity; BD: bulk density; SRP: Soil resistance penetration; TP: total porosity; MiP: microporosity; MaP: Macroporosity; MWD: Mean weight diameter; BG: Beta-glucosidase activity.

Suplemmentary table 2 – SMAF scores (SQI7) for SOC, pH, P, BD, EC, BG, K and WFPS.

Treatments	SOC		pH		P		BD		EC		BG		K		WFPS	
T1	0,828	c	0,910	a	0,969	a	0,609	b	0,860	a	0,162	a	0,336	c	0,910	a
T2	0,831	c	0,943	a	0,982	a	0,494	b	0,930	a	0,158	b	0,425	c	0,933	a
T3	0,850	b	0,948	a	0,970	a	0,562	b	0,984	a	0,142	b	0,489	b	0,894	a
T4	0,835	b	0,956	a	0,958	a	0,578	b	0,943	a	0,151	b	0,548	b	0,930	a
T5	0,847	b	0,915	a	0,973	a	0,541	b	0,894	a	0,109	b	0,546	b	0,910	a
T6	0,883	a	0,900	a	0,989	a	0,977	a	1,000	a	0,043	c	0,943	a	0,949	a

Means followed by same letters did differ each other by the scott-knott test ($p<0,05$). SOC: soil organic carbon; P: phosphorus;BD: bulk density; EC: electrical conductivity; BG: beta-glucosidadse activity; K: potassium; WFPS: water filled pore space.