



Article Sustainability Assessment after Twenty Years of Sewage Sludge Application on Calcareous Soil Following N or P Criterion

Francesc Camps-Sagué¹, Bernat Lavaquiol², Àngela Dolores Bosch-Serra^{2,*}, Maria Gabriela Molina³ and Francesc Domingo-Olivé¹

- ¹ IRTA Mas Badia, Agricultural Experimental Station Mas Badia, E-17134 La Tallada d'Empordà, Spain; francesc.camps@irta.cat (F.C.-S.); francesc.domingo@irta.cat (F.D.-O.)
- ² Department of Chemistry, Physics, Environmental Sciences and Soil, University of Lleida, E-25198 Lleida, Spain; bernat.lavaquiol@udl.cat
- ³ Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba X5000HUA, Argentina; gabmolin@agro.unc.edu.ar
- * Correspondence: angela.bosch@udl.cat

Abstract: Sewage sludge is a valuable source of nutrients when applied to the soil. Research on its agricultural use has been focused on chemical parameters to prevent heavy metal buildup. However, soil quality includes a wider spectrum of indicators. Our aim was to evaluate the impacts of sludge application on the biological and physical soil properties of calcareous soil when sludge is applied in maize monoculture using fertilization dosage criteria determined by N input or soil P thresholds. A control based on mineral fertilization was also included. After 20 years, no differences were found in the biological indicators: earthworm and soil oribatid mite abundances. Five oribatid species were identified, but three predominated: *Acrotritia ardua americana, Oribatula (Zygoribatula) excavata* and *Tectocepheus sarekensis*. The latter two showed a maximum number of individuals five months after maize stalks were buried in soil, independent of fertilization treatment. Sludge significantly increased water-stable aggregates (up to 30%), but there was no difference in macroporosity (apparent pore diameter > 30 μ m). Under irrigated calcareous soil, sewage sludge applied as fertilizer is a sustainable option, independent of the threshold criterion (N or P) used. Nevertheless, as the P threshold criterion allows for reduced P inputs, it is more sustainable over the long term.

Keywords: aggregate stability; earthworm; mineral fertilization; organic fertilizer; oribatid mites; soil porosity

1. Introduction

The agricultural systems of developed countries are open systems [1]. They are not autonomous, which means that they are highly dependent on external inputs such as fertilizers. Some agricultural systems are close to urban areas, which might be an important source of sewage sludge that may be used as fertilizer. Sewage sludge (SS) is a subproduct of wastewater treatment, and urban organic waste recycling and valorization, based on the principles of circular economy, is an interesting option for the European Union [2,3]. The application of SS to soil is an economic-nutrient source, as organic fertilizers may partially or totally replace mineral fertilizers without yield reduction [4]. Fertilization using sewage sludge might therefore be a practice that could increase sustainability.

In Spain, according to the National Sludge Registry, 1,200,000 dry matter tons of SS are generated annually [5], of which ca. 80% is suitable for agronomic valorization [6]. At the national level, 71% of the available SS is applied in agricultural fields [5]. Sludge provides nutrients and organic matter [7], although its composition varies depending on the wastewater source and the provided treatment [8]. Nevertheless, the use of SS is associated with different ecotoxicity risks, for example, pharmaceutical compounds [9] or antibiotic resistance genes [10]. Although regulations have been set up to reduce potential



Citation: Camps-Sagué, F.; Lavaquiol, B.; Bosch-Serra, À.D.; Molina, M.G.; Domingo-Olivé, F. Sustainability Assessment after Twenty Years of Sewage Sludge Application on Calcareous Soil Following N or P Criterion. *Sustainability* **2024**, *16*, 2304. https://doi.org/10.3390/su16062304

Academic Editor: Agostina Chiavola

Received: 14 February 2024 Revised: 6 March 2024 Accepted: 6 March 2024 Published: 11 March 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). impacts, these only restrict the heavy metal contents [11,12] and the maximum amount of nitrogen (170 kg N ha⁻¹) allowed to be applied in nitrate-vulnerable areas [13]. However, the average sludge N:P ratio is 2.45, while in the maize plant the N:P ratio is 10 [14], and so if fertilization is carried out according to the maximum allowed sludge nitrogen criterion, the phosphorus in the soil may increase considerably under maize monoculture. For this reason, there is growing interest in a fertilization strategy that follows a sludge phosphorus criterion based on the amount of available soil P rather than the maximum allowed N rate from organic materials. This approach aims to mitigate the risk of P accumulation in the soil and potential P leaching [15]. It might also contribute to the circular economy [16] and to the sustainability of agricultural systems as P resources are scarce and phosphate rock is a non-renewable resource [17].

The use of sludge as a fertilizer has been extensively studied as a nutrient source [18–20] and so has the transfer of nutrients to the crop [21]. Considerably less effort, however, has been invested in describing the impact of sludge applications on soil quality [22], especially in calcareous soils where heavy metals are more easily immobilized due to their high soil pH. Apart from its chemical properties [21], soil quality can also be assessed based on its biological and physical properties. Earthworms respond to changes in soil chemistry and agricultural practices, and so their abundance is considered to be a good environmental indicator [23]. Additionally, variables such as species number are also recognized as reliable indicators of environmental conditions [4]. Another bioindicator is the oribatid community. Oribatids are one of the main groups of decomposers. In fact, a high correlation exists between soil organic matter and the specific diversity of oribatids [24-27]. They are sensitive to soil degradation and have a robust correlation with soil functioning. The latter explains why they are useful for assessing soil status [24,28]. Apart from the organic matter content, oribatid communities are modified by other soil properties, including the disturbance level and heavy metal concentration [29,30]. Oribatid species can also be negatively affected by the addition of treated SS to soil [31]. Among the physical soil properties, the characterization of the soil pore system [32], changes in soil structure features [33], and aggregate stability are of interest. However, only long-term experiments are able to provide the required precision for aggregate stability studies [34].

A long-term experiment was developed in an irrigated region in the northeast of Spain where soils are calcareous. The general aim was to analyze the effects of sewage sludge application on the biological and physical soil properties, compared with mineral fertilization. Maize (*Zea mays* L.) was established annually as a monoculture. The experiment had three fertilizer treatments: sludge applied following the nitrogen criteria (maximum allowed N rate from organic fertilizers), sludge applied following the phosphorus criteria (maximum threshold of Olsen-P soil level not to be exceeded), and mineral fertilization (based on extensionist recommendations in the area). We hypothesized that sludge applied following the phosphorus criterion may be a more agronomic and environmentally sustainable option than the nitrogen criterion when physical and biological soil quality parameters are introduced as indicators, although yields might not be affected in the medium term.

The main objectives of this work were to assess both the changes in the biological soil quality indicators (earthworm abundance, oribatid mite species abundance, and number of individuals for the different identified mite species) and the changes in the physical soil quality indicators (soil porosity and soil aggregate stability) in a maize monoculture following sewage sludge fertilization for a period of 20 years. Furthermore, from the obtained results, the aim includes a recommendation about the best fertilization treatment to apply from the point of view of physical and biological soil quality while sustaining yields.

2. Materials and Methods

2.1. Experimental Site and Experimental Design

The experiment was established in 1996 in La Tallada d'Empordà (Girona, NE Spain) and maintained from then on. The coordinates of the site are 42°05′37″ N, 03°06′39″ E, and the altitude is 17 m a.s.l. The area has a dry Mediterranean climate. The annual

average temperature for the period from 1999 to 2018 was 14.9 °C, with high average summer temperatures of 22.6 °C. The annual average precipitation in the same period was 646 mm year⁻¹, with most rain falling during the months of September, October and November. The annual average reference evapotranspiration (FAO Penman–Monteith equation) was 976.9 mm year⁻¹ for the same period. In 2019, annual rainfall and reference evapotranspiration were 549 mm and 1056 mm, respectively. Irrigation is needed for summer crops in the region, and furrow irrigation is widespread in the area. From the 2015 cropping season, the experimental field was under a high-frequency irrigation system (exudation belt lines).

The soil was classified as an Oxyaquic Xerofluvent, and the family particle-size class was coarse-loamy [35]. The soil was deep (>1.2 m), non-saline, and calcareous without stones. Soil bulk density was 1565 kg m⁻³ for the first 0.3 m depth interval. The upper layer (0–0.2 m) had a sandy loam texture, a pH (potentiometry, water 1:2.5; *w*:*v*) of 8.2, and an electrical conductivity (1:5, 25 °C, *w*:*v*) of 0.18 dS m⁻¹. Equivalent calcium carbonate (Bernard's calcimeter method) was 140 g kg⁻¹.

The experiment included three fertilization treatments, which were distributed according to a randomized block design with four replicates. The annual treatments included a mineral fertilization treatment as a control (MIN) and two sludge treatments where doses followed a threshold sludge nitrogen criterion (SNC) or a soil threshold phosphorus criterion (SPC). The MIN treatment corresponded to the mineral fertilization followed by farmers in the area. In the 2019 cropping season, 100 kg ha⁻¹ of N, P₂O₅ and K₂O were applied on the 10 April 2019. At topdressing (25 June 2019), they were complemented by 70 kg N ha⁻¹. In the SNC treatment, sludge was also applied on the 10 April 2019 at a rate of 170 kg N ha⁻¹ with a mineral supplementation of 100 kg K₂O ha⁻¹. The SPC followed the SNC treatment, but when soil Olsen-P levels exceeded 40–60 mg P kg⁻¹, no sludge was applied the following season, and instead, only minerals N and K were applied as in the MIN treatment. Thus, from 2006 to 2009 and in 2014, no sludge was applied in SPC. In 2019, sludge was applied in the SPC and in the SNC treatments. The plots receiving the sludge application had an area of 41 m² (9.6 m long and 4.25 m wide). The control plots had an area of 81.6 m² (9.6 m long and 8.5 m wide).

After the 2018 harvest (10 October 2018), as is customary every year, the maize stalks were chopped and incorporated into the soil through ploughing. Also, after sludge application in March 2019, a disk-plough operation was carried out on all plots included in the experimental field, following the common practice in the area. Sowing was performed on the 12 April 2019 at a density of 80,000 seeds ha⁻¹. Seeds were treated with the flowable concentrate Sonido (Bayer Crop Science) containing Tiacloprid (400 g L⁻¹). The maize variety was P1570Y (Pioneer). The preemergence herbicide was Camix 3.5 (4% w/v Mesotrione, 40% w/v S-metolachlor) from Syngenta. Irrigation began on the 7th June and finished the 16th September. Total applied water was 3936 m³ ha⁻¹. Historical average yields were close to 14 Mg ha⁻¹ and there were no differences between treatments. The 2019 harvest (10 October 2019) had average yields (±standard deviation) of 13,779 (±1209) kg ha⁻¹. No differences between treatments were observed (Table A1).

2.2. Field Samplings and Measurements

Every season, before the organic fertilizer application, a sludge sample was taken and analyzed in the laboratory in order to adjust the rate to 170 kg N ha⁻¹, as it was conducted in March 2019 (Table 1). Total N was quantified by the Kjeldahl digestion and distillation method [36] and total carbon by ignition at 550 °C. Heavy metals and P and K in sludges were analyzed from microwave digested samples (UNE-EN 16174:2012) [37] with aqua regia (3:1, *v:v*, HCl:HNO3), and they were quantified using inductively coupled plasma mass spectrometry (UNE-EN 16171) [38].

	Fertilization Treatment		Sewage	
Soil and SS Properties	MIN	SPC	SNC	Sludge
pH (1:2.5, soil)/(1:5, SS)	8.3	8.1	8.1	8.3
Electrical conductivity (1:5 w/v ; dS m ⁻¹)	0.15	0.16	0.16	1.62
Organic matter (g kg $^{-1}$)	12	15	16	69.2
Mineral N (NO ₃ $^{-}$ N, UV; mg kg $^{-1}$)	9.7	12.3	14.7	-
Total N (Kjeldahl, g kg $^{-1}$)	-	-	-	61.2
Phosphorous (Olsen/total in SS; mg P kg $^{-1}$)	14.9	39.2	57.1	19.5
Potassium (NH ₄ AcO 1 N/total in SS; mg K kg ^{-1})	99	96	87	3.04
Cadmium (UNE-EN 16174:2012/total; mg Cd kg $^{-1}$)	< 0.5	< 0.5	< 0.5	1.16
Copper (UNE-EN 16174:2012/total; mg Cd kg $^{-1}$)	44.3	52.4	52.5	668
Chrome (UNE-EN 16174:2012/total; mg Cr kg ^{-1})	18.0	19.5	17.4	38
Mercury (UNE-EN 16174:2012/total; mg Hg kg $^{-1}$)	< 0.4	< 0.4	< 0.4	0.71
Nickel (UNE-EN 16174:2012/total; mg Ni kg $^{-1}$)	21.5	17.7	16.6	27
Lead (UNE-EN 16174:2012/total; mg Pb kg $^{-1}$)	17.8	18.6	19.1	68
Zinc (UNE-EN 16174:2012/total; mg Zn kg $^{-1}$)	72.2	82.2	82.8	1208

Table 1. Soil physicochemical characteristics ¹ (October 2018) of the upper layer (0–0.2 m) according to the treatment: Mineral (MIN), sludge N criterion (SNC) or soil P criterion (P). Composition of the sewage sludge (SS), applied in March 2019 on dry matter basis, is included.

¹ In soil, properties are specified according to the fertilization treatments applied during the previous 20 years.

Before the 2019 maize cropping season, the soil was resampled with an auger (composite samples from each treatment) on the 5 October 2018 for a more detailed chemical characterization (Table 1). Soil samples were immediately transferred to the laboratory in polyethylene bags protected from sunlight. These soil samples were stored at 4 °C until nitrate content was analyzed by ionic chromatography [39] after nitrate extraction with demineralized water. Afterwards, soil samples were dried at room temperature and ground to 2 mm. Soil pH was determined in aqueous solution using a 1:2.5 (soil:water) ratio, salinity (electrical conductivity, EC) by conductimetry (1:5), oxidizable organic carbon by [40], available P content by the Olsen method (sodium bicarbonate-extractable P at pH 8.5 [41], and exchangeable K by extraction with ammonium acetate 1 N (pH = 7) [42] and determined by atomic absorption spectrophotometry. Soil heavy metals were analyzed following the same procedures used in sludges.

Earthworms were quantified from topsoil samples obtained on the 10 October 2018 (at maize harvest) and on the 3 July 2019 from the four replicates of each treatment. Monoliths (0.2 m long, 0.2 m wide, and 0.2 m deep) were excavated from the surface. A total of 24 monolithic samples were studied. Earthworms were separated from the soil following the sampling protocol proposed by the Spanish standard UNE-ISO-23611-1 [43]. Earthworms were extracted manually from the monoliths, washed with warm water, stored for 24 h in ethanol (70%) and then fixed with formaldehyde. The extracted individuals were quantified for abundance considering the whole earthworm body, and if it was cut, only the fore part (i.e., with the head) was counted.

Oribatid mite identification was performed in soil samples obtained on the 10 October 2018, 6 March 2019 and 3 July 2019 from three replicates of each treatment. In each replicate, a composite sample was obtained from 3 sampled points, for a total of 36 composite samples studied. Sampling was performed with soil cores (6 cm in diameter) from 0 to 5 cm in soil depth, as oribatids mainly accumulate close to the surface [44]. They were extracted with a Berlese-Tullgren funnel over a period of 12 days, and they were directly preserved in 70% ethanol. For identification at species level, they were immersed in lactic acid.

Soil porosity and microstructure were characterized from undisturbed soil surface prisms (0.15 m deep, 0.15 m wide and 0.2 m long) excavated on the 3 October 2018 from each replicate (12 prisms). From each prism, a horizontal, thin section (30 μ m thick) was obtained following the procedure established by [45]. The 12 soil sections were scanned, and a central area (43.9 mm high and 81.4 mm wide) was processed with the software

ImageJ[®] (IJ1.46r) [46] to obtain digital binary images. These binarized images were used to quantify porosity. Pores with an apparent diameter (APD) < 30 μ m were discarded.

Aggregate stability was evaluated from topsoil samples (0–0.15 m depth) obtained on the 3 October 2018 from each replicate of the three treatments (12 samples). Firstly, soil aggregate stability was quantified according to the mean weight diameter (MWD) or the aggregate-size distribution after fast wetting. The MWD was determined following the guidelines provided by [47] and modifications outlined by [48]. Secondly, the evaluation of water-stable aggregates (WSA) was performed. The WSA were obtained following the procedure described by [49] but using dry aggregates, as this allows better evaluation of the slaking disaggregation effect under Mediterranean conditions [50].

2.3. Statistical Analysis

The absolute abundance of earthworms was assessed using a Generalized Linear Mixed model (GLMM) with a Binomial Negative distribution appropriate for count data and utilizing a square root (sqrt) link function. Overdispersion was scrutinized, and model assumptions were validated through the examination of residual plots. The GLMM incorporated a random intercept for the block-sampling time interaction and treated treatment and sampling time as fixed effects. The statistical analyses were conducted using R (version 3.6.2) with the 'Ime4' package [51].

Oribatid abundance was analyzed using PROC MIXED from SAS version 9.4 for Windows (SAS Institute Inc., Cary, USA). A square root transformation was applied to abundance data to make the variation more uniform. The statistical model contained a fixed effect for treatment and sampling time and a block as a random effect. Treatment least squares means were computed using the LSMEAN. Also from SAS, the GLMM procedure was used to analyze differences in the number of mites present and associated with the identified species in each sampling. Only species with more than five individuals present for each treatment and sampling were included in the analysis.

An analysis of variance (ANOVA analysis) was used to test the effect of fertilization treatments on soil porosity, MWD and WSA. Means separation was performed according to the Duncan multiple range test for the $\alpha = 0.05$ probability level.

3. Results

The soil chemical characterization of the field plots (according to the historical fertilization treatments) and the chemical properties of the sludge applied in 2019 are summarized in Table 1. Soil parameters reflect some characteristic trends under sludge fertilization: the increase of organic matter, mineral N, total Cu, total Zn and available P.

Earthworm abundance in soil samples showed high variability, and it was neither affected by sampling date or by treatment (Table A2). Average earthworm abundance (±standard deviation) was 71 (±38) and 35 (±28) individuals m⁻² (in 0.2 m deep) in October 2018 and in July 2019, respectively. In treatments, earthworm abundance average (±standard deviation) was 34 (±38), 69 (±57) and 64 (±89) individuals m⁻² (in 0.2 m soil depth) for MIN, SPC and SNC, respectively.

The oribatid abundance varied significantly with time (Table A3) but without differences between treatments. In March, abundance was significantly higher than in October and July which showed similar values (Figure 1).



Figure 1. Oribatid abundance changes in the cropping season (October, March and July samplings) for the different fertilization treatments: mineral (MIN), soil phosphorous criterion using sludges (SPC) and sludge N criterion (SNC). Vertical bars indicate \pm one corrected sample standard deviation (n = 3). Different capital letters on the columns indicate significant differences between time samplings (*p* < 0.05).

Three main species were identified (Figure 2): *Acrotritia ardua americana* (Ewing, 1909), *Oribatula (Zygoribatula) excavata* Berlese, 1916 and *Tectocepheus sarekensis* Trägårdh, 1910. Another two species were present: *Scapheremaeus corniger* (Berlese, 1908) that can be considered an outsider as only one individual was found, and *Zetomimus (Protozetomimus) acutirostris* (Mihelčič, 1957) with less than a total of five individuals present in each treatment and in different samplings.



Figure 2. Accumulated abundance (over three samplings) of oribatid mite species for a sampled area (84 cm²) and for the different fertilizer treatments: mineral (MIN), soil phosphorous criterion using sludges (SPC) and sludge N criterion (SNC).

The presence of the two main species, *O. excavata* and *T. sarekensis*, varied with time (Table A4); they followed the total abundance trend (Figure 1). The highest values were in the March sampling period. There were no differences between the other sampling periods (October and July). The presence of *A. ardua* did not show significant differences with time or between treatments. As a result, in March, the presence of *A. ardua* was significantly

lower than that of *O. excavata* and *T. sarekensis* (Table A5). The values in March 2019 were 1336, 4637 and 6013 individuals m⁻² for *A. ardua*, *O. excavata* and *T. sarekensis*, respectively. In the thin-section analysis, prominent porosity features were observed (Figure 3).



Figure 3. Representative scanned images from the thin sections used for microstructure description, according to the different treatments: MIN (mineral fertilization), SPC (soil P criterion using sludges), and SNC (sludge N criterion).

In thin sections, it was also observed that MIN treatment did not have much pedality, as demonstrated by the fact that it did not develop many aggregates. The structure was massive (with a few cracks between aggregates) with small pores. Some wildlife activity was detected associated with biopores (faunal channels and chambers). Vesicles and cavities were observed as isolated pores. The SPC treatment showed cracks and packing pores between aggregates, which were very irregular. The SNC treatment resulted in greater pedality than SPC, although cracks and packing pores between aggregates were similar

to the SPC treatment. However, no differences were found in total meso-macroporosity (>30 μ m) (Table A6). Total porosity in the studied area was 49.5%, 47.0% and 40.3% for MIN, SPC and SNC, respectively.

Micromorphological observations matched with the aggregate stability results. The sludge additions significantly increased MWD and WSA values when compared with the mineral treatment (Table A7, Figure 4).



Figure 4. Mean weight diameter (MWD) and water stable aggregates (WSA) for the different treatments: MIN (mineral fertilization), SPC (soil P criterion using sludges), and SNC (sludge N criterion). Average columns with different letters are significantly different according to the Duncan multiple range test for the α = 0.05 probability level. Vertical lines show the standard deviation.

4. Discussion

As maize yields were above 13 Mg ha⁻¹, the agricultural management of the experimental field can be considered highly productive [52], regardless of the fertilizer treatment applied. It is noteworthy that high yields are obtained when the annual maximum rate restriction of 170 kg ha⁻¹ of N from organic origin, which applies in European nitrate vulnerable areas [53], is respected in SNC and SPC treatments. High yields can also be attained when Olsen-P soil levels are maintained below (*c*. half values) the upper soil threshold of 80 mg P kg⁻¹ established by the Catalan government in Spain [54].

For loamy soil, the fertility level based on the available P in MIN treatment was acceptable (normal level) [9]. However, values of available P were very high under sludge treatments [9], mainly when the SNC criterion was followed. Available K was low in all treatments [9]. Heavy metal concentrations in the soil were far below the upper Spanish threshold levels [12,55]. In sludge, heavy metal concentrations were also below the upper Spanish thresholds [12,55].

Earthworm abundance was low. The number of recorded individuals was half that recorded in calcareous soils under cereal rainfed systems, where figures have been shown to oscillate between 100 and 176 individuals m^{-2} [4]. Low soil moisture in October (to allow maize harvester traffic) or high air temperatures in July (average of 24.8 °C with a maximum of 34.6 °C) were conditions that might have constrained earthworm activity [56]. In fact, the above-mentioned authors indicate that the end of wintertime and the beginning of springtime are the most favorable for earthworm activity. The addition of organic matter is considered to be one of the management aspects that most affects the abundance of earthworms, as organic matter is a source of food for worms and allows population growth [28]. However, no differences were found between treatments, probably because the average soil organic matter content was low (12–16 g kg⁻¹, Table 1). Nevertheless, earthworm activity through bioturbation was visible in soil-thin sections (Figure 3).

The sampling period with the highest oribatid mite abundance was in March (Tables A3 and A4; Figure 1), irrespective of the treatment. Variations among sampling dates were expected, given that the overall abundance of oribatid mites is known to fluctuate throughout the year and show a peak related to their reproductive cycle, i.e., from egg deposition to the adult stage [57]. In March, the average oribatid mite abundance was noteworthy (12,302 individuals m⁻²). This figure is double the recorded values from

areas with similar rainfall under grassy arable fallows [58]. Three main oribatid species were found. This result means low diversity. In fact, the density and diversity of oribatids have been shown to decrease as the intensification of agriculture increases [59,60]. The presence of the eurytopic species T. sarekensis is important because Tectocepheidae are commonly found in cultivated soils and heavily disturbed areas [61]. Tectocepheus sarekensis and A. ardua have been identified as suitable indicators for negative agricultural management impacts [62]. Moreover, T. sarekensis is also suitable for assessing the degree of ecosystem vulnerability [30]. Their presence might serve as an early warning for the health of the maize monoculture ecosystem. In this experiment, it is noteworthy that no significant differences were associated with treatments; instead, differences were observed only among sampling dates. This absence of differences between treatments in the presence of *T. sarekensis* could indicate the sustainability of the applied rates. Furthermore, Oribatula (Zygoribatula) excavata usually establishes an abundant community where animal droppings are applied at agronomic rates when N overfertilization is avoided [63]. No decreasing trend in the abundance of these species at high sludge rates (SNC) was observed, indicating that they were not affected by P overfertilization.

In terms of microstructure, the increased pedality of SNC compared to SPC (Figure 3) could be interpreted as a saturation effect resulting from the highest sludge rate, which prevented the structure from developing further. Some authors support the idea that the potential benefits of SS for soil structure are limited to their use within a certain dose range [64]. No significant differences between treatments in terms of the percentage of total porosity (>30 µm) were found. Total porosity includes biopores, cracks and pores between aggregates. Additionally, it should be noted that mesoporosity (apparent pore diameter between 30 and 70 μ m) and macroporosity (apparent pore diameter > 70 μ m) are linked to soil water drainage. Consequently, the potential movement of water and nutrients due to bypass flow was not significantly altered. The isolated vesicles and pores, predominantly observed in the MIN treatment, are a characteristic of soils with a low organic matter content [65]. Therefore, sludge applications also contributed to the improvement of this aspect. Furthermore, the circularity observed in some pores in the MIN treatment (Figure 3) is associated with some fauna channels but not with structure improvement (few cracks between aggregates). In contrast, a greater abundance of rounded aggregates was observed in SNC and SPC compared to MIN (Figure 3). The addition of sewage sludge, regardless of the dose applied, also resulted in a significant improvement in structural stability evaluated as MWD and WSA, which coincides with previous findings [66].

5. Conclusions

The application of sewage sludge, regardless of the dosage criterion (annual incorporation of sludge equivalent to 170 kg N ha⁻¹ or the avoidance of sludge use when available Olsen-P soil exceeded 40–60 mg P kg⁻¹ in the upper layer), improved soil physical quality in comparison to full mineral fertilization. Greater amounts of stable aggregates and the development of a less massive soil microstructure when sewage sludge is applied support a better soil physical quality. Furthermore, no negative impacts of the different fertilization models on biological quality were observed, as evidenced by the presence of earthworms, the abundance of oribatid mites and the variety of species present. The species distribution showed no differences between fertilization treatments; instead, differences were linked to a sampling period. Thus, in calcareous soil and based on the presented results, both dosage and sewage sludge criteria can be followed as fertilization schedules in irrigated maize. However, the soil P threshold criterion, as an option for saving P inputs, appears to be the best sustainable fertilizer strategy.

Author Contributions: Conceptualization F.C.-S., F.D.-O. and À.D.B.-S.; methodology, À.D.B.-S.; investigation, B.L.; formal analysis, M.G.M. and B.L.; data curation, B.L. and M.G.M.; writing—original draft preparation, B.L.; writing—review and editing, À.D.B.-S.; project administration, F.C.-S.; funding acquisition, F.C.-S. and F.D.-O. All authors have read and agreed to the published version of the manuscript.

Funding: Spanish Ministry of Economy and Competitiveness and the Spanish National Institute for Agricultural Research and Experimentation (MINECO-INIA) through the project RTA2017-88-C3-3. The experimental site maintenance was funded by IRTA-Mas Badia and the Department of Climate Action, Food and Rural Agenda from Generalitat de Catalunya, Catalonia, Spain.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the corresponding author upon reasonable request.

Acknowledgments: The authors thank E. González for field support and M. Antúnez and S. Porras for their laboratory support. R.M. Poch is fully acknowledged for her advice related to micromorphology. M.M. Boixadera-Bosch is acknowledged for her edition support.

Conflicts of Interest: The authors declare no conflicts of interests.

Appendix A

Table A1. Analysis of variance of maize yields (kg ha⁻¹ on dry matter basis) in the 2019 harvest.

Source	df	F-Value	<i>p</i> -Value
Between blocks	3	0.52	0.6812
Between treatments	2	3.46	0.0999

Table A2. Analysis of deviance (Type II Wald chi-square tests) of earthworm count in soil monoliths (0.2 m long, 0.2 m wide, 0.2 m deep) for the three treatments and the two sampling dates.

Source	df	Chi-Square	<i>p</i> -Value
Between time samplings	1	1.49	0.221
Between treatments	2	2.34	0.310
Interaction sampling:treatment	2	4.37	0.112

Table A3. Analysis of variance of oribatid mite abundance (square root transformation of individuals m^{-2}) for the three treatments and the three sampling dates.

Source	df	dendf	F-Value	<i>p</i> -Value
Between time samplings	2	18	10.27	0.001
Between treatments	2	18	0.58	0.570
Interaction sampling:treatment	4	18	0.14	0.963

Table A4. Analysis of variance for the presence (counts of individuals in each sample of 28 cm²) of three oribatid mite species for the three fertilization treatments and for the three sampling dates.

Species df Source df	16		A. ardua		O. excavata		T. sarekensis	
	ar	dendf	F-Value	<i>p</i> -Value	F-Value	<i>p</i> -Value	F-Value	<i>p</i> -Value
Between time samplings	2	16	1.77	0.202	5.33	0.022	3.86	0.043
Between treatments	2	16	0.65	0.533	0.15	0.865	0.44	0.649
Interaction sampling:treatment	4	16	0.42	0.795	0.04	0.997	0.44	0.779

Source	df	dendf	F-Value	<i>p</i> -Value
Between species	2	16	5.42	0.016
Between treatments	2	16	0.07	0.930
Interaction species:treatment	4	16	0.96	0.457

Table A5. Analysis of variance of the presence (counts of individuals in each sample of 28 cm²) of three oribatid mite species in March 2019 and for the three fertilization treatments.

Table A6. Analysis of variance of porosity percentage in October 2018.

Source	df	Sum of Squares	F-Value	<i>p</i> -Value
Between blocks	3	35.583	0.32	0.811
Between treatments	2	183.167	2.47	0.165

Table A7. Analysis of variance of the mean weight diameter and water stable aggregates in October 2018.

C	16	Mean Weight Diameter			Water Stable Aggregates		
Source	ar	Sum of Squares	F-Value	<i>p</i> -Value	Sum of Squares	F-Value	<i>p</i> -Value
Between blocks	3	0.00042	2.43	0.1635	116.49	2.78	0.132
Between treatments	2	0.00411	35.29	0.0005	570.04	20.41	0.002

References

- Drinkwater, L.E. Systems Research for Agriculture. Innovative Solutions to Complex Challenges; SARE Outreach Publications: Brentwood, MD, USA, 2016; pp. 1–23. Available online: https://www.sare.org/wp-content/uploads/Systems-Research-for-Agriculture.pdf (accessed on 13 January 2024).
- Meyer-Kohlstock, D.; Schmitz, T.; Kraft, E. Organic waste for compost and biochar in the EU: Mobilizing the potential. *Resources* 2015, 4, 457–475. [CrossRef]
- 3. Sharma, B.; Sarkar, A.; Singh, P.; Pratap Singh, R. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. *Waste Manag.* 2017, 64, 117–132. [CrossRef]
- Valdez, A.S.; Bosch-Serra, A.D.; Yagüe, M.R.; Poch, R.M.; Puigpinós, E. Earthworm community and soil microstructure changes with long-term organic fertilization. *Arch. Agron. Soil Sci.* 2019, 66, 957–970. [CrossRef]
- 5. Instituto Nacional de Estadística. Estadística Sobre el Suministro y Saneamiento del Agua. Serie 2000–2020. Available online: https://www.ine.es/jaxi/Datos.htm?path=/t26/p067/p01/serie/l0/&file=01008.px#!tabs-tabla (accessed on 1 March 2024).
- Nadal Rocamora, I.; Gondim Porto, C.; Platero Alonso, L.; Navarro-García, F. Uso de lodos de depuradora en agricultura: Patógenos y resistencias a antibióticos. *Rev. Salud Ambient.* 2015, 15, 113–120.
- Gobierno de España. Real Decreto 506/2013, de 28 de Junio, Sobre Productos Fertilizantes. Available online: https://www.boe. es/eli/es/rd/2013/06/28/506/con (accessed on 13 February 2024).
- Ministerio para la Transición Ecológica y el Reto Demográfico. Caracterización de Los Lodos de Depuradoras Generados en España (NIPO 770-10-256-5). 2009. Available online: https://www.miteco.gob.es/es/calidad-y-evaluacion-ambiental/ publicaciones/lodos.html (accessed on 13 February 2024).
- 9. Martínez, E.; Andrades, M.S. Fertilidad del Suelo y Parámetros Que la Definen, 4th ed.; Universidad de la Rioja, Servicio de Publicaciones: Logroño, Spain, 2022.
- Urra, J.; Alkorta, I.; Mijangos, I.; Epelde, L.; Garbisu, C. Application of sewage sludge to agricultural soil increases the abundance of antibiotic resistance genes without altering the composition of prokaryotic communities. *Sci. Total Environ.* 2019, 647, 1410–1420. [CrossRef] [PubMed]
- 11. European Economic Community. Council Directive 86/278/EEC of 12 June 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture. *OJEU L* **1986**, *181*, 6–12.
- 12. Gobierno de España. Real Decreto 1310/1990, de 29 de Octubre, Por el Que se Regula la Utilización de los Lodos de Depuración en el Sector Agrario. Available online: https://www.boe.es/eli/es/rd/1990/10/29/1310/con (accessed on 13 February 2024).
- Gobierno de España. Real Decreto 47/2022, de 18 de Enero, Sobre Protección de Las Aguas Contra la Contaminación Difusa Producida Por Los Nitratos Procedentes de Fuentes Agrarias. Available online: https://www.boe.es/eli/es/rd/2022/01/18/47/ con (accessed on 13 February 2024).
- Lal, R. Plant nutrients: Sufficiency and requirements. In *Encyclopedia of Soil Science*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2017; pp. 1735–1739.

- 15. Ortiz, C.; Pierotti, S.; Molina, M.G.; Bosch-Serra, À.D. Soil fertility and phosphorus leaching in irrigated calcareous soils of the Mediterranean region. *Environ. Monit. Assess.* **2023**, *195*, 1376. [CrossRef]
- Stamm, C.; Binder, C.R.; Frossard, E.; Haygarth, P.M.; Oberson, A.; Richardson, A.E.; Schaum, C.; Schoumans, O.; Udert, K.M. Towards circular phosphorus: The need of inter- and transdisciplinary research to close the broken cycle. *Ambio* 2022, *51*, 611–622. [CrossRef] [PubMed]
- Van Kauwenbergh, S.J. World Phosphate Rock Reserves and Resources; International Fertilizer Development Center (IFDC) Publications: Muscle Shoals, AL, USA, 2010. Available online: https://pdf.usaid.gov/pdf_docs/Pnadw835.PDF (accessed on 10 February 2024).
- McGrath, S.P.; Cegarra, J. Chemical extractability of heavy metals during and after long-term applications of sewage sludge to soil. *Eur. J. Soil Sci.* 1992, 43, 313–321. [CrossRef]
- 19. Moreno, J.L. Uso de Composts de Lodo de Depuradora Para la Mejora de la Calidad de los Suelos de Zonas Áridas: Efecto de su Contaminación Metálica. Ph.D. Thesis, Universidad de Murcia, Murcia, Spain, 1997.
- Sloan, J.J.; Dowdy, R.H.; Dolan, M.S.; Linden, D.R. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils. J. Environ. Qual. 1997, 26, 966–974. [CrossRef]
- 21. Zaragüeta, A.; Enrique, A.; Virto, I.; Antón, R.; Urmeneta, H.; Orcaray, L. Effect of the long-term application of sewage sludge to a calcareous soil on its total and bioavailable content in trace elements, and their transfer to crop. *Minerals* **2021**, *11*, 356. [CrossRef]
- 22. Simoes-Mota, A.; Poch, R.M.; Enrique, A.; Orcaray, L.; Virto, I. Soil quality assessment after 25 years of sewage sludge vs. mineral fertilization in a calcareous soil. *Land* **2021**, *10*, 727. [CrossRef]
- 23. Paoletti, M.G. The role of earthworms for assessment of sustainability and as bioindicators. *Agric. Ecosyst. Environ.* **1999**, 74, 137–155. [CrossRef]
- 24. Andrés, P.; Mateos, E. Soil mesofaunal responses to post-mining restoration treatments. Appl. Soil Ecol. 2006, 33, 67–78. [CrossRef]
- 25. Noble, J.C.; Whitford, W.G.; Kaliszweski, M. Soil and litter microarthropod populations from two contrasting ecosystems in semi-arid eastern Australia. *J. Arid. Environ.* **1996**, *32*, 329–346. [CrossRef]
- Sadaka, N.; Ponge, J.F. Soil animal communities on holm oak forests: Influence of horizon, altitude and year. *Eur. J. Soil Biol.* 2003, 39, 197–207. [CrossRef]
- Schneider, K.; Migge, S.; Norton, R.A.; Scheu, S.; Langel, R.; Reineking, A.; Maraun, M. Trophic niche differentiation in soil microarthropods (Oribatida, Acari): Evidence from stable isotope ratios (15N/14N). *Soil Biol. Biochem.* 2004, 36, 1769–1774. [CrossRef]
- Doran, J.W.; Zeiss, M.R. Soil health and sustainability: Managing the biotic component of soil quality. *Appl. Soil Ecol.* 2000, 15, 3–11. [CrossRef]
- 29. Gergócs, V.; Hufnagel, L. Application of oribatid mites as indicators (review). Appl. Ecol. Environ. Res. 2009, 7, 79–98. [CrossRef]
- Feketeová, Z.; Mangová, B.; Čierniková, M. The soil chemical properties influencing the oribatid mite (Acari; Oribatida) abundance and diversity in coal ash basin vicinage. *Appl. Sci.* 2021, 11, 3537. [CrossRef]
- 31. Andrés, P. Ecological risks of the use of sewage sludge as fertilizer in soil restoration: Effects on the soil microarthropod populations. *Land Degrad. Dev.* **1999**, *10*, 67–77. [CrossRef]
- 32. Pagliai, M.; Vignozzi, N. The soil pore system as an indicator of soil quality. Adv. GeoEcol. 2002, 35, 69–80.
- Pagliai, M.; Vignozzi, N.; Pellegrini, S. Soil structure and the effect of management practices. Soil Tillage Res. 2004, 79, 131–143. [CrossRef]
- 34. Peterson, G.A.; Lyon, D.J.; Fenster, C.R. Valuing long-term field experiments: Quantifying the scientific contribution of a long-term tillage experiment. *Soil Sci. Soc. Am. J.* **2012**, *76*, 757–765. [CrossRef]
- 35. Soil Survey Staff. Keys to Soil Taxonomy, 13th ed.; USDA-Natural Resources Conservation Service: Washington, DC, USA, 2022.
- McGill, W.B.; Figueiredo, C.T. Chapter 22: Total Nitrogen. In Soil Sampling and Methods of Analysis; Carter, M., Ed.; CRC Press: Boca Raton, FL, USA, 1993; pp. 201–207.
- AENOR. UNE-EN 16174; Sludge, Treated Biowaste and Soil—Digestion of Aqua Regia Soluble Fractions of Elements. Asociación Española de Normalización y Certificación: Madrid, España, 2012.
- AENOR. UNE-EN 16171; Sludge, Treated Biowaste and Soil—Determination of Elements Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Asociación Española de Normalización y Certificación: Madrid, España, 2016.
- United States Environmental Protection Agency. Method 9056A. Determination of Inorganic Anions by Ion Chromatography. 2007. Available online: https://www.epa.gov/sites/default/files/2015-12/documents/9056a.pdf (accessed on 10 February 2024).
- 40. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
- 41. Pansu, M.; Gautheyrou, J. Chapter 29: Phosphorus. In *Handbook of Soil Analysis. Mineralogical, Organic and Inorganic methods;* Pansu, M., Gautheyrou, J., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 2003; p. 809.
- Hendershot, W.H.; Lalande, H.; Duquette, M. Ion exchange and exchangeable cations. In Soil Sampling and Methods of Analysis, 2nd ed.; Carter, M.R., Gregorich, E.G., Eds.; Canadian Society of Soil Science, CRC Press and Taylor and Francis Group: Boca Raton, FL, USA, 2008; pp. 197–206.
- AENOR. UNE-ISO 23611-1; Calidad del Suelo. Muestreo de Invertebrados del Suelo. Parte 1: Cribado Manual y Extracción con Formol de Lombrices. Asociación Española de Normalización y Certificación: Madrid, España, 2009.

- 44. Marshall, V.G. Seasonal and vertical distribution of soil fauna in a thinned and urea-fertilized Douglas fir forest. *Can. J. Soil Sci.* **1974**, *54*, 491–500. [CrossRef]
- 45. Benyarku, C.A.; Stoops, G. *Guidelines for Preparation of Rock and Soil Thin Sections and Polished Sections. Quaderns DMACS 33*; Departament de Medi Ambient i Ciències del Sòl, Universitat de Lleida: Lleida, Spain, 2005.
- 46. Rueden, C.T.; Schindelin, J.; Hiner, M.C.; DeZonia, B.E.; Walter, A.E.; Arena, E.T.; Eliceiri, K.W. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinf.* 2017, *18*, 529. [CrossRef] [PubMed]
- 47. Le Bissonnais, Y. Experimental study and modelling of soil surface crusting processes. Catena Suppl. 1990, 17, 13–28.
- Amezketa, E.; Singer, M.J.; Le Bissonnais, Y. Testing a new procedure for measuring water-stable aggregation. *Soil Sci. Soc. Am. J.* 1996, 60, 888–894. [CrossRef]
- 49. Kemper, W.D.; Rosenau, R.C. Aggregate stability and size distribution. In *Methods of Soil Analysis. Part I Physical and Mineralogical Methods. Agronomy Monograph* 9, 2nd ed.; Klute, A., Ed.; ASA and SSSA: Madison, WI, USA, 1986; pp. 425–442.
- 50. Domingo-Olivé, F.; Bosch-Serra, À.D.; Yagüe, M.R.; Poch, R.M.; Boixadera, J. Long term application of dairy cattle manure and pig slurry to winter cereals improves soil quality. *Nutr. Cycl. Agroecosyst.* **2016**, *104*, 39–51. [CrossRef]
- 51. Bates, D.; Mächler, M.; Bolker, B.; Walker, S. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 2015, 67, 1–48. [CrossRef]
- 52. Berenguer, P.; Santiveri, F.; Boixadera, J.; Lloveras, J. Nitrogen fertilisation of irrigated maize under Mediterranean conditions. *Eur. J. Agron.* **2009**, *30*, 163–171. [CrossRef]
- 53. European Union. Council Directive 91/676/EEC, of 12 December 1991, Concerning the Protection of Waters Against Pollution Caused by Nitrates from Agricultural Sources. Official Journal of the European Communities L375. Available online: https: //eur-lex.europa.eu/eli/dir/1991/676/oj (accessed on 10 February 2024).
- 54. Generalitat de Catalunya. Decree 153/2019, of 3rd July, on the Management of the Soil Fertilization and Manure, and the Implementation of the Action Program in Vulnerable Areas to Nitrate Pollution from Agricultural Sources. Available online: https://portaljuridic.gencat.cat/ca/document-del-pjur/?documentId=853461&validity=1957101&traceability=02&language=ca (accessed on 13 February 2024).
- 55. Ministerio de la Presidencia, Justicia y Relaciones con Las Cortes. Real Decreto 1051/2022, de 27 de Diciembre, Por el Que se Establecen Normas Para la Nutrición Sostenible en Los Suelos Agrarios. Available online: https://www.boe.es/buscar/doc.php? id=BOE-A-2022-23052 (accessed on 13 February 2024).
- Pérès, G.; Cluzeau, D.; Hotte, H.; Delaveau, N. Earthworms. In *Bioindicators. Biological Tools for Soil Quality Assessment*; ADEME, Ed.; Agence de l'Environnement et de la Maîtrise de l'Energie: Rennes, France, 2014.
- 57. Wehner, K.; Heethoff, M.; Brückner, A. Seasonal fluctuation of oribatid mite communities in forest microhabitats. *PeerJ* 2018, *6*, e4863. [CrossRef]
- 58. Wissuwa, J.; Salamon, J.A.; Frank, T. Oribatida (Acari) in grassy arable fallows are more affected by soil properties than habitat age and plant species. *Eur. J. Soil Biol.* **2013**, *59*, 8–14. [CrossRef]
- 59. Badieritakis, E.G.; Fantinou, A.A.; Emmanouel, N.G. A qualitative and quantitative comparison of mite fauna between bifenthrintreated and non-pesticide treated alfalfa hay fields in Central Greece. *Hell. Plant Prot. J.* **2020**, *13*, 29–41. [CrossRef]
- Bedano, J.C.; Cantú, M.P.; Doucet, M.E. Influence of three different land management practices on soil mite (Arachnida: Acari) densities in relation to a natural soil. *Appl. Soil Ecol.* 2006, 32, 293–304. [CrossRef]
- 61. Behan-Pelletier, V.M. Oribatid mite biodiversity in agroecosystems: Role for bioindication. *Agric. Ecosyst. Environ.* **1999**, 74, 41–423. [CrossRef]
- Bosch-Serra, À.D.; Molina, M.G.; González-Llinàs, E.; Boixadera-Bosch, R.R.; Martínez, B.; Orobitg, J.; Mateo-Marín, N.; Domingo-Olivé, F. Oribatid mites in different Mediterranean crop rotations fertilized with animal droppings. *Exp. Appl. Acarol.* 2023, 90, 185–202. [CrossRef] [PubMed]
- 63. Bosch-Serra, À.D.; Padró, R.; Boixadera-Bosch, R.R.; Orobitg, J.; Yagüe, M.R. Tillage and slurry over-fertilization affect oribatid mite communities in a semiarid Mediterranean environment. *Appl. Soil Ecol.* **2014**, *84*, 124–139. [CrossRef]
- Simões-Mota, A.; Virto, I.; Poch, R.M. Effects of long-term sewage sludge application to a calcareous soil structure. *Soil Use Manag.* 2022, *38*, 1693–1704. [CrossRef]
- Malobane, M.E.; Nciizah, A.D.; Bam, L.C.; Mudau, F.N.; Wakindiki, I.I.C. Soil microstructure as affected by tillage, rotation and residue management in a sweet sorghum-based cropping system in soils with low organic carbon content in South Africa. *Soil Tillage Res.* 2021, 209, 104972. [CrossRef]
- 66. Sort, X.; Alcañiz, J.M. Effects of sewage sludge amendment on soil aggregation. Land Degrad. Dev. 1999, 10, 3–12. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.