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Article

A Fertilisation Strategy Combining Mineral Fertiliser and Biosolid Improves Long-Term Yield and Carbon Storage in a Calcareous Soil

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Abstract: At a strategic moment for agricultural soils, which are expected to contribute to climate change mitigation through carbon storage while safely feeding a growing world population, the fertiliser strategies used will be key. In a calcareous soil with extensive rainfed agricultural use and straw removal, different fertiliser strategies were evaluated with the aim of determining their effects on crop yield, nitrogen agronomic efficiency, and the storage of organic carbon and total nitrogen in the soil. Different doses of mineral fertiliser, expressed as kg of mineral nitrogen ha⁻¹ year⁻¹ (0, 60, 120, 180, and 240 nitrogen fertilising units (NFUs)), were applied to plots with and without biosolid amendment. The biosolid, applied at a rate of 40 Mg ha⁻¹ every 3 years for 18 years, complied with national and European regulations to be applied on agricultural soil. The use of combined fertilisation reduced the amount of mineral fertiliser applied between 33 and 67% and the total fertiliser units between 7 and 40%, while maintaining similar yields to the reference mineral fertilisation (180 NFUs). These results could be related to a higher nitrogen agronomic efficiency in the combined fertilisation treatments that do not exceed the total NFUs required by the crop. Combined fertilisation was also an effective fertiliser technique to store total nitrogen and organic carbon in the soil. However, compared to the reference mineral fertilisation (180 NFUs), no significant changes in the soil organic carbon were observed, probably due to the crop management method in which the straw is removed and to higher gas emissions. Our results support the need to assess the efficacy of each agricultural technique at local scales in order not to overestimate or underestimate the potential of each agricultural technique to store soil organic carbon.

Keywords: long term; calcareous soil; biosolid; mineral fertiliser; fertiliser strategy; extensive crops; yields; nitrogen agronomic efficiency; soil organic carbon



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1. Introduction

Agricultural soil management is a key aspect of restoring soil fertility and helping to mitigate climate change through the storage of soil organic carbon (SOC) [1–4]. However, the response of agricultural soils to different management techniques can be highly variable in relation to SOC storage [5–9]. Some agronomic techniques, such as fertilisation, minimum tillage, or the application of organic residues, tend to increase SOC [6,10–14]. However, the efficacy of these techniques is determined by the edaphoclimatic characteristics of the agricultural area [12,15–17]. Therefore, it is essential to define the local soil and climatic conditions and the agricultural management of the field in order to apply the techniques that increase SOC. Part of the difficulty in increasing SOC lies in its cycling rate, which

is closely related to the total N in the soil. Thus, the amount of C and N, as well as their ratio, will determine various biological and abiotic processes such as the emission of greenhouse gases (GHGs) or a priming effect that will directly influence the SOC stock [18]. In addition, organic N determines the stability of SOC to a great extent by influencing soil fertility [18,19]. Therefore, knowledge of the soil total nitrogen (STN) will be essential to understand the possible dynamics of SOC.

At the same time, agricultural soils must be able to safely feed a continuously growing world population [20–23]. This challenge comes at a time where unexpected state movements, such as wars, can lead to a rise in the price of inputs as a consequence of low supply [24,25]. To meet this challenge, increasing the nitrogen agronomic efficiency (NAE), defined as the amount of additional grain harvested per kilogram of N applied to a grain crop, is positioned as a key strategy. Some nitrogen fertiliser management strategies can positively alter the NAE (the use of rotations, organic fertilisers, or slow-release mineral fertilisers or the fractionation of mineral doses) [26–28]. Therefore, replacing fertiliser strategies that can decrease the NAE [29–31] with fertiliser techniques that maintain or increase the NAE would allow a reduction in the use of mineral fertilisers and contribute to more economical and environmentally sustainable agriculture.

Therefore, to simultaneously increase the SOC and STN and feed the world, different fertiliser techniques will be a key strategy. On the one hand, the use of mineral nitrogen fertilisers has favoured food production [29,30,32] and has been able to increase SOC and STN stocks by favouring aerial and root biomass growth [32–36], while avoiding a loss of organic matter through mineralisation [37–40]. However, the inadequate and excessive use of these mineral fertilisers can cause environmental damage such as soil acidification, leaching, the emission of a high amount of GHGs, and a loss of SOC [41–46]. On the other hand, the use of organic amendments such as biosolids from urban wastewater treatment plants has demonstrated multiple benefits while favouring the circular economy. These benefits can be agronomic, by contributing to crop yields [47–49], or edaphic, contributing to an increase in SOC and STN, among other benefits [36,50,51]. However, the poor usage of biosolids can have disadvantages such as the accumulation of trace metals and/or emerging pollutants [52–54], as well as GHG emissions [6,55–57].

A fertilisation strategy that could be interesting as opposed to “monotype” fertilisation (organic only vs. mineral only) is the use of balanced combined fertilisation (CF) created by mixing an organic amendment and mineral fertiliser. With the use of CF, several studies have observed a simultaneous increase in yield, STN, and SOC, contributing to valuable agricultural objectives [58–63]. However, to correctly employ combined fertilisation, it would be essential to know the efficiency of the biosolid to provide N to the crop annually to accordingly adjust the amount of mineral fertiliser applied. Not accounting for this substitute value could have consequences, such as either not reaching the N needs of the crop or providing higher amounts of available N than necessary.

From this vantage point, we hypothesised that the use of a biosolid would reduce the amount of mineral fertiliser required and increase organic carbon and total nitrogen in the soil without hindering the cereal yield potential. The overall objective of this study was to assess the efficacy of alternative fertilisation strategies combining a mineral fertiliser and a biosolid in a calcareous soil. Specifically, we focused on the effects of the fertilisation strategy in four relevant agronomic parameters: (I) the crop yield, (II) the soil organic carbon stock, (III) the soil total nitrogen stock, and (IV) nitrogen agronomic efficiency. Our work is motivated by the need to define effective, locally grounded strategies to simultaneously increase food production and maintain a healthy soil organic carbon and nitrogen content.

2. Materials and Methods

2.1. Site and Experimental Design

The sampling site belongs to a long-term trial that began in September 2003 and that has continued without interruption for 18 years, up to the time of sampling. It is located on an experimental field of the Commonwealth of Pamplona, in the municipality

of Arazuri in the region of Navarre, in Northwestern Spain. According to the Papadakis climate classification, the climate corresponds to the humid temperate Mediterranean group (Meth), with a temperature, precipitation, and potential evapotranspiration (according to Thornthwaite) of 12.9 °C, 771 mm, and 696.7 mm, respectively [64]. The soil of the plot is a Calcareous Cambisol [65] and the tilled horizon (0–30 cm) has a silt–clay loam texture, pH 8.5, 16% carbonates, and no slope, and has been used for rainfed farming for decades. Crop management is conventional, with annual mouldboard tillage, the application of agrochemicals according to the needs of the season, the incorporation of stubble, and the removal of straw. A three-year cereal–cereal–fallow rotation, typical of this rainfed area, is followed. The trial has a factorial design, with elementary plots of 54 m² (9 m long by 6 m wide) and 4 replications for each fertiliser treatment. The different fertiliser treatments were formed with the combination of two factors: (I) *BioSolid*: application or not of a biosolid (2 levels) and (II) *Mineral Nitrogen (Nmin)*: proportions of the recommended dose (NFUs, kg of mineral nitrogen ha⁻¹ year⁻¹) to satisfy the local and annual extraction needs of the crop (6 levels).

The levels of the factor *BioSolid* correspond to an application of 40 Mg ha⁻¹ of a biosolid every 3 years or the lack of this application (*BioSolid* 0 Mg ha⁻¹). This dose was calculated in 1992, at the opening of the Arazuri urban wastewater treatment plant, following the restrictive regulation of 250 NFUs in areas that are not considered vulnerable to nitrate pollution without taking into account the N efficiency for the year of application [66,67]. Biosolid application was carried out with a 3.5 m wide spreader trailer and the biosolid was mixed in the first 30 cm of the soil in the following 48 h after the application. Subsequently, seeding was carried out. Since the beginning of the trial, the treatments receiving 40 Mg ha⁻¹ every 3 years have received a cumulative amount of 240 Mg ha⁻¹ of the biosolid spread over 6 applications. This amount corresponds to 15.8 Mg ha⁻¹ accumulated organic carbon applied to the soil over the 18 years (2.6 Mg ha⁻¹ per application) according to analytical data (Table 1). The factor *Nmin* was computed as the proportion of mineral N applied by mineral fertiliser corresponding to 0, 1/3, 2/3, 3/3, and 4/3 of 100% of the recommended NFUs for the implanted crop. This recommended NFU dose was calculated considering a restitution dose of nitrogen extractions for an average production of 6 Mg ha⁻¹ of winter wheat in the area, corresponding to the average productivity of the agroclimatic zone. This quantity corresponds to 180 NFUs in the case of rainfed wheat, a crop grown in the sampling year, thus obtaining the technoeconomic optimum. Therefore, the NFUs applied by mineral fertiliser in each treatment were 0, 60, 120, 180, and 240. The NFUs corresponding to each fertiliser treatment were applied at two different times depending on the phenological stage of the crop. The first 60 NFUs were applied coinciding with the phenological stage of tillering, and the remaining dose was applied during the stem elongation stage. The mineral nitrogen fertiliser used was a mixture of 46% urea and 21% ammonium sulphate in a ratio of 70:30. The treatments that did not receive the biosolid also received a bottom dressing of 60 P₂O₅ fertiliser units in super format at 45%.

From the combination of both factors, 10 treatments were obtained, one being the control treatment (*BioSolid*: No; *Nmin*: 0 NFUs) and the other the reference treatment (*BioSolid*: No; *Nmin*: 180 NFUs). The treatments were named according to the amount of NFUs in the mineral fertiliser format they contained, and in the case of the biosolid treatments, a “+” was added. The studied fertiliser treatments, as well as their nomenclature, are shown in detail in Table 2.

2.2. Biosolid Composition and Amendment Properties

The Arazuri urban wastewater treatment plant receives wastewater from the City of Pamplona and its metropolitan area, a population of approximately 335,000 [68]. The wastewater is subjected to a primary phase (pretreatment and settling), followed by a secondary or biological phase (aeration basin and secondary settling). The resulting sludge receives anaerobic digestion, homogenisation, and dewatering. From this point on, it is called a biosolid. The use of this biosolid as an organic amendment in agriculture complies

with national and European regulations [69,70]. For this reason, its physical and chemical properties are monitored by the competent official authorities from the beginning of the trial. Regarding the concentration of heavy metals in the biosolid and their possible transfer to the soil and crops, a study was carried out on the joint experimental plot amended with this same biosolid [71]. Table 1 shows that these properties remained relatively unchanged during the study period.

Table 1. Physicochemical properties of the biosolid used in this study in 2003 (start of the experiment) and 2018 (application) together with the analytical methods used. Mean annual values \pm standard deviation. In the table, SOC and STN stand for soil organic carbon and soil total nitrogen, respectively.

Physical-Chemical Properties	2003	2018	Analysis Method
General parameters			
pH	8.2 \pm 0.03	8.2 \pm 0.03	Soil pH in water 1:5 [72]
Electrical conductivity ($\mu\text{s cm}^{-3}$)	1749 \pm 24	1795 \pm 28	Diluted extracts 1:5 [73]
Dry matter (%)	19.0 \pm 0.35	18.1 \pm 0.4	Direct calcination at 540 °C [74]
Organic carbon (% of dry matter)	38.7 \pm 0.7	36.4 \pm 1.1	Walkley–Black wet oxidation [75]
C:N	6.0 \pm 0.1	5.5 \pm 0.1	SOC: STN ratio
Fertilizing elements (% of dry substance)			
N total	6.5 \pm 0.2	6.6 \pm 0.2	Kjeldahl digestion and distillation [76]
Phosphorus (P_2O_5)	7.1 \pm 0.2	5.6 \pm 0.2	Microwave digestion + ICP-MS [77,78]
Potassium (K_2O)	0.6 \pm 0.0	0.6 \pm 0.1	Microwave digestion + ICP-MS [77,78]
Nitrogen Fertiliser Units (NFUs)	493	478	Estimation from total N
Trace metals (mg kg^{-1} dry weight)			
Cadmium (Cd)	*	0.88 \pm 0.09	Microwave digestion + ICP-MS [77,78]
Copper (Cu)	303 \pm 13.2	187 \pm 11	Microwave digestion + ICP-MS [77,78]
Nickel (Ni)	51 \pm 2.2	32.1 \pm 0.77	Microwave digestion + ICP-MS [77,78]
Lead (Pb)	71 \pm 4.2	39.0 \pm 1.2	Microwave digestion + ICP-MS [77,78]
Zinc (Zn)	1408 \pm 71	874 \pm 38	Microwave digestion + ICP-MS [77,78]
Mercury (Hg)	*	0.003 \pm 0.003	Microwave digestion + ICP-MS [77,78]
Chromium (Cr)	180 \pm 8.1	58.3 \pm 3.2	Microwave digestion + ICP-MS [77,78]

*—Not analysed.

Table 2. Nitrogen fertiliser units (NFUs) contributed to the crop in the sampling year by biosolid (BS), mineral fertiliser (Nmin), and total (sum of BS and Nmin).

Fertiliser Treatment	BS	Nmin	Total	Nomenclature
0 Mg ha ⁻¹ of BS + 0 NFUs of mineral fertiliser	0	0	0	0 NFUs
0 Mg ha ⁻¹ of BS + 60 NFUs of mineral fertiliser	0	60	60	60 NFUs
0 Mg ha ⁻¹ of BS + 120 NFUs of mineral fertiliser	0	120	120	120 NFUs
0 Mg ha ⁻¹ of BS + 180 NFUs of mineral fertiliser	0	180	180	180 NFUs
0 Mg ha ⁻¹ of BS + 240 NFUs of mineral fertiliser	0	240	240	240 NFUs
40 Mg ha ⁻¹ of BS + 0 NFUs of mineral fertiliser	48	0	48	0+ NFUs
40 Mg ha ⁻¹ of BS + 60 NFUs of mineral fertiliser	48	60	108	60+ NFUs
40 Mg ha ⁻¹ of BS + 120 NFUs of mineral fertiliser	48	120	168	120+ NFUs
40 Mg ha ⁻¹ of BS + 180 NFUs of mineral fertiliser	48	180	228	180+ NFUs
40 Mg ha ⁻¹ of BS + 240 NFUs of mineral fertiliser	48	240	288	240+ NFUs

The effectiveness of the biosolid to supply mineral N to the crop used in this trial was 30% for the 1st year, 20% for the 2nd year, and 10% for the 3rd year after its application. These coefficients were calculated from previous trials on cereal crops managed by the Instituto Navarro de Tecnologías e Infraestructuras Agroalimentarias (INTIA) [79].

2.3. Nitrogen Fertiliser Units in Each Fertiliser Treatment

The NFUs available to the crop of each treatment can be seen in Table 2. The NFUs of the biosolid in the year of sampling (3rd year after the last application) were calculated using the biosolid efficiencies mentioned in the previous section (10%) and the initial biosolid fertilisation dose applied (478 NFUs, Table 1). The total NFUs of the treatments were calculated by adding the NFUs contributed by the biosolid (0 or 48) and the NFUs added using mineral fertilisation (0, 60, 120, 180, and 240).

2.4. Soil Sampling and Analytical Determinations

The soil samples were taken in early summer 2021, before harvest, 18 years after the start of the trial, and 3 years after the last biosolid application. Disturbed soil samples were collected from each treatment and replicated at a 0–30 cm depth with an Edelman auger. For each treatment and replicate, a sample composed of three randomly located subsamples was collected, avoiding the edges of the plots. The three subsamples were mixed in a polythene bag and transferred for processing, protected from sunlight. Undisturbed samples with 100 cm³ rings were also taken from each plot for bulk density determination. In the laboratory, the disturbed samples were air-dried at room temperature and ground to a size of 2 mm for further analysis. The undisturbed samples were oven-dried at 105 °C for 48 h.

2.4.1. Crop Yield and Nitrogen Agronomic Efficiency

Wheat, of the variety “Filón” [80], was harvested a few days after the sample collection. The yields were assessed by harvesting a 13.5 m² (1.5 m × 9 m) area from each plot. The harvester used was a self-propelled HALDRUP C-65 microplot harvester (Haldrup, Denmark) with automated yield and quality records and a 1.5 m cut. The yields, expressed as Mg ha⁻¹, were calculated with reference to 12% grain moisture.

The nitrogen agronomic efficiency (NAE, kg ha⁻¹) was computed as follows:

$$NAE = \frac{(Y_T - Y_C)}{F} \quad (1)$$

where Y_T is the grain yield of the treatment (kg ha⁻¹), Y_C is the yield of the control treatment (*BioSolid*: No; *Nmin*: 0 NFUs) (kg ha⁻¹), and F is the total NFUs of the treatment (kg ha⁻¹, Table 2).

2.4.2. Soil Organic Carbon and Soil Total Nitrogen Analysis

The soil organic carbon content was determined according to the Walkley–Black wet oxidation method [75] due to the high concentration of carbonates in the soil. The total nitrogen in the soil was determined following the Dumas combustion method. The scaling of both determinations to a soil mass per area corresponding to a soil depth of 30 cm was conducted by using the bulk density of each microplot.

2.5. Statistical Analysis

Statistical analysis was conducted using R software [81]. Nitrogen agronomic efficiency was analysed using one-way analysis of variance (ANOVA) for the determination of the significance between treatments. The soil carbon content, soil nitrogen content, and grain yield were analysed using a full-factorial, two-way ANOVA for the determination of the significance between *Nmin* and *BioSolid* treatments. The “lm” and “anova” functions of the Stats package were used for this analysis. A Type III Sum of Squares provided by the “Anova” function of the car package [82] was assumed when data were not fully balanced due to a missing value. Post hoc analysis using the “HSM.test” function from the agricolae package [83] with the appropriate setting of the “unbalanced” flag and the default significance level of 0.05 were used to separate the significant treatment combinations. The normality and homoscedasticity of the residuals were assessed using the Shapiro–Wilk normality test provided by the function “shapiro.test” of the stats package [81] and the “leveneTest” function of the car package [82].

The data were also analysed by means of a one-way analysis of covariance (ANCOVA) using the *Nmin* levels as the numerical covariate and the *BioSolid* treatments as the factor. With this approach, the significance of the interaction term allows assessing if the slope of the data (the increase in the dependent variable per mineral NFU added) differs in the presence of an organic amendment, which would highlight differences in the efficiency of fertilisation to store carbon or nitrogen in the soil.

The ANOVA and ANCOVA tables obtained in this contribution can be seen in the supplementary material file (Tables S1–S7), where interested readers are referred.

3. Results

3.1. Crop Yield

ANOVA found a significant effect of the factors *BioSolid* (p -value < 0.001) and *Nmin* (p -value < 0.001), as well as the interaction (p -value < 0.001), on the grain yield of the crop. The significance of the interaction term was due to the saturation of the grain yield at higher fertilisation levels (Figure 1). Without the addition of organic fertiliser (*BioSolid*: No), the maximum grain yield was attained from the *Nmin* level of 180 NFUs. Nonetheless, when the organic fertiliser was present (*BioSolid*: Yes), attaining the maximum grain yield required a lower dose of *Nmin*: 120 NFUs. The mean yields of the treatments allowing the achievement of maximum yield production (letter *a* in Figure 1) were not statistically distinguishable ($\alpha = 0.5$), irrespective of whether the organic fertiliser was added or not.

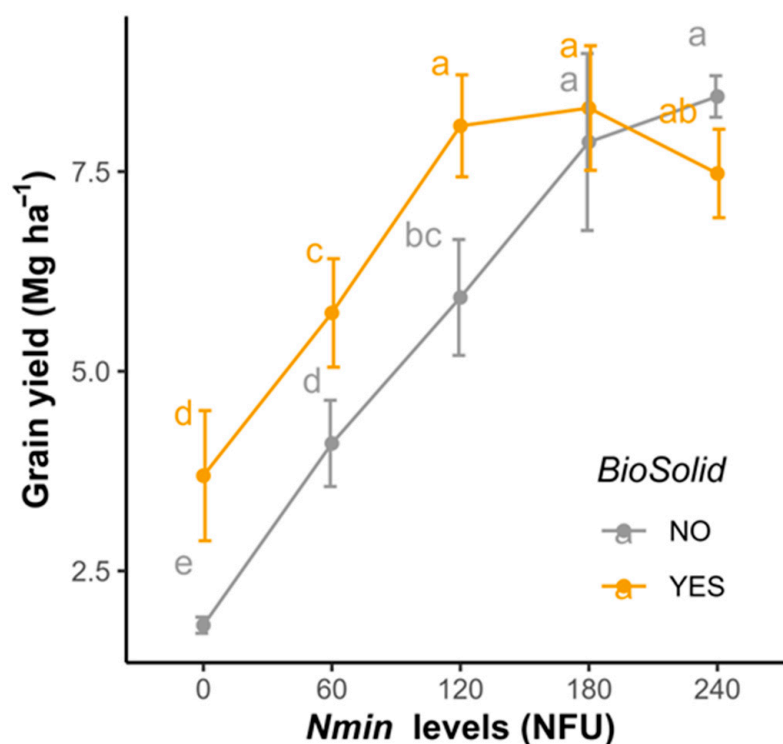


Figure 1. Grain yield (Mg ha^{-1}) as a function of the *Nmin* and *BioSolid* levels. Treatments with the same letter are not significantly different ($\alpha = 0.5$). Error bars show 1 standard deviation of 4 replicated data.

The ANCOVA analyses were conducted including the *Nmin* levels 0, 60, and 120 NFUs, which showed a linear relationship with the grain yield. A significant effect of *BioSolid* (p -value < 0.001) and *Nmin* (p -value < 0.001) was found. The interaction between the factor and the covariate was shown to be not significant (p -value < 0.65), suggesting that, before the yield saturation, the increase in yield per NFU was not statistically different using a pure mineral (*BioSolid*: No) or a mixed organic–mineral amendment strategy (*BioSolid*: Yes).

3.2. Nitrogen Agronomic Efficiency

The ANOVA found significant differences between the fertiliser treatments (p -value < 0.01). As can be seen in Table 3, the highest NAE was observed in the fertiliser treatments adding 60 NFUs (*BioSolid*: No; *Nmin*: 60), 60+ NFUs (*BioSolid*: Yes; *Nmin*: 60), and 120+ NFUs (*BioSolid*: Yes; *Nmin*: 120). The lowest NAE was obtained in the 240+ NFUs (*BioSolid*: Yes; *Nmin*: 240) treatment.

Table 3. Nitrogen agronomic efficiency (NAE) as a function of fertiliser treatment. The table shows the mean value \pm standard deviation of each *BioSolid* and *Nmin* combination. Treatments with the same letter are not significantly different ($\alpha = 0.5$).

<i>Nmin</i>	<i>BioSolid</i>	
	No	Yes
0	*	31.5 \pm 11.8 (ab)
60	37.9 \pm 8.9 (a)	36.2 \pm 7.2 (a)
120	34.2 \pm 6.7 (ab)	37.2 \pm 4.2 (a)
180	33.6 \pm 6.0 (ab)	28.4 \pm 3.8 (ab)
240	27.6 \pm 1.3 (ab)	19.6 \pm 1.7 (b)

*—Not calculated.

3.3. Soil Organic Carbon

The ANOVA found a significant effect of the factors *BioSolid* (p -value < 0.001) and *Nmin* (p -value = 0.02), but not of their interaction (p -value < 0.92), on the soil organic carbon content. In general terms, the soil organic carbon stocks found when the organic fertiliser was used (*BioSolid*: Yes) were similar to the ones obtained at the higher *Nmin* levels (180 and 240 NFUs) (Figure 2).

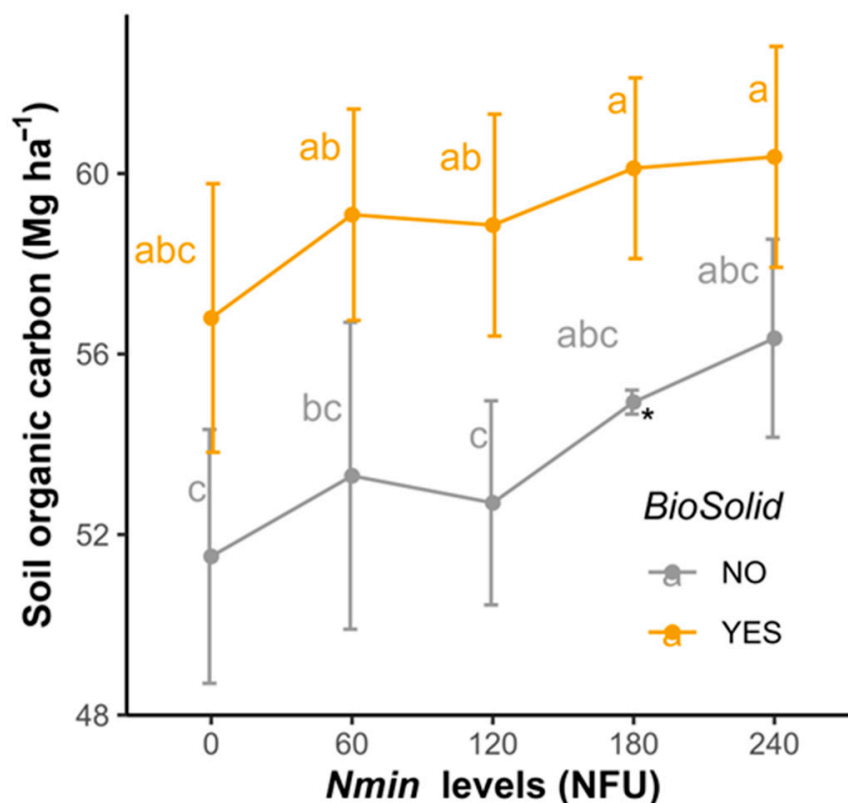


Figure 2. Soil organic carbon (Mg ha⁻¹) as a function of the *Nmin* and *BioSolid* levels. Treatments with the same letter are not significantly different ($\alpha = 0.5$). Error bars show 1 standard deviation of 4 replicated data. Symbol “*” denotes a group with one missing value.

The ANCOVA suggested a highly significant effect of *BioSolid* (p -value < 0.001) and *Nmin* (p -value < 0.001) to explain the soil organic carbon stocks. The interaction between the factor and the covariate was not significant (p -value < 0.55), suggesting that the increase in soil organic carbon per mineral NFU added was not statistically different, irrespective of whether a pure mineral amendment strategy (*BioSolid*: No) or a mixed organic–mineral approach (*BioSolid*: Yes) was assumed.

3.4. Soil Total Nitrogen

The ANOVA suggested a highly significant effect of the factor *BioSolid* (p -value < 0.001) to explain the observed values of total nitrogen. The *Nmin* factor was not significant at a significance level of 0.05 (p -value = 0.11) and the *BioSolid*:*Nmin* interaction was clearly not significant (p -value = 0.96). Therefore, only the application of the biosolid significantly increased the soil total nitrogen content. Taking this into account, the soil total nitrogen was significantly higher when the organic fertiliser was used (*BioSolid*: Yes) (Figure 3).

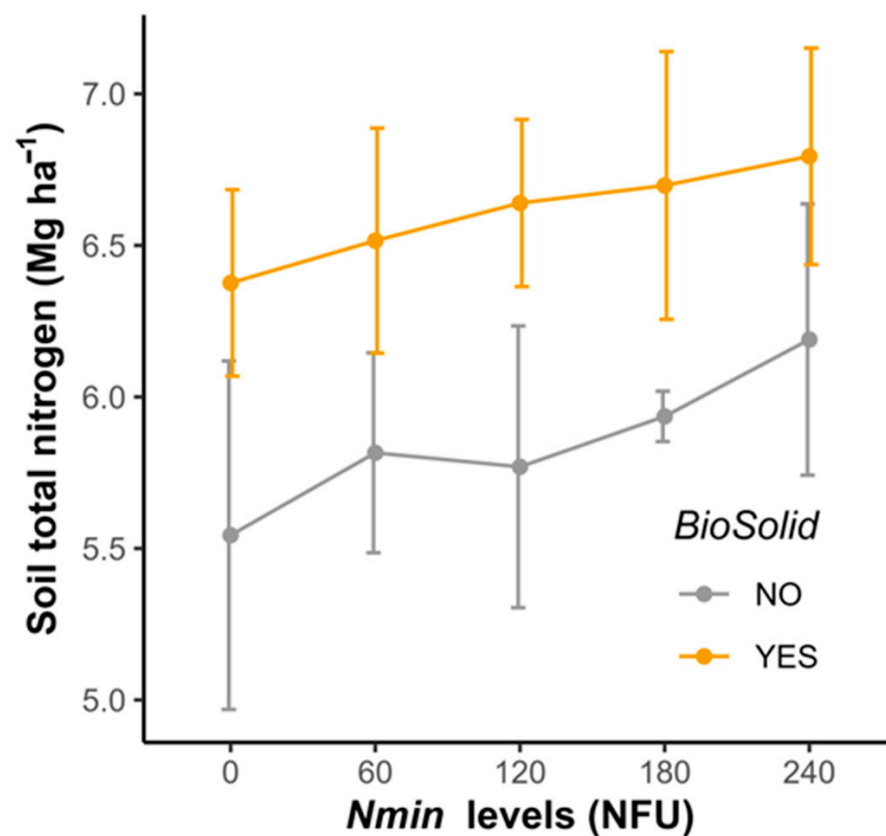


Figure 3. Soil total nitrogen (Mg ha^{-1}) as a function of the *Nmin* and *BioSolid* levels. Error bars show 1 standard deviation of 4 replicated data.

A significant effect of both *BioSolid* (p -value < 0.001) and *Nmin* (p -value < 0.01) to explain the measured total soil nitrogen could, nonetheless, be detected using ANCOVA, which suggests that both *Nmin* and *BioSolid* may indeed explain the soil total nitrogen recorded in the study. A not-significant effect of the *BioSolid*:*Nmin* interaction (p -value = 0.62) was supported by ANCOVA as well. This suggests that there was no statistical difference between the accumulation rate of soil total nitrogen as the mineral nitrogen dose increased, irrespective of whether the organic fertiliser was added (*BioSolid*: Yes) or not (*BioSolid*: No).

4. Discussion

4.1. Crop Yield and Nitrogen Use

Yield saturation was reached earlier with the application of *BioSolid*. As shown in Figure 1, the maximum yield was reached with the 120+ treatment (168 total NFUs, Table 2), and from the *Nmin* 120 NFUs onwards, the yield stagnated. In contrast, when the *BioSolid* was not applied, the maximum yield was reached at 180 NFUs. The ANCOVA showed no difference in the yield increase per unit of *Nmin* input independently of *BioSolid*; that is, the yield increase rate before the grain yield saturated was the same with and without the biosolid.

The 120+ NFUs treatment (*BioSolid*: Yes; *Nmin*: 120) provided the N necessary to cover the maximum productive needs of the crop, as did the 180 NFUs treatment. In this combined fertilisation (CF) treatment, the biosolid is likely to have provided progressively inorganic N released by the mineralization and nitrification processes [59,84–87]. This was probably also favoured by increased soil total nitrogen (STN) stocks motivated by the CF used (Figure 3) [63,88]. This supply of inorganic N can also be partially explained by an increased biological activity favoured by the biosolid. In that respect, some authors [84] found an increase in root metabolism induced by microbial activity three years after the biosolid application, which ensured the maximum wheat grain yield.

The grain yields observed in our study were comparable to the historical average production of the agricultural region (Region III in [89]). In this area, approximately 180–240 NFUs are used, and its grain yield is around 5.5–6 Mg ha⁻¹ [90–92]. In the study year, the 120+ NFUs and 180 NFUs treatments obtained yields of 7.5 Mg ha⁻¹, with the average for the area that year being 5.8 Mg ha⁻¹ [93]. Focusing on the latter, even the 60+ treatment (108 total NFUs, Table 2) would have been sufficient to achieve the average yield of the region (Figure 1). Decreased mineral NFU needs to achieve maximum yields have been reported by similar studies performing annual applications of organic amendments including biosolids [59,61–63,94]. Our contribution extends their findings by demonstrating (i) the long-term effect of these effects and (ii) that not only minerals, but also the total NFUs, can be reduced with the use of CF.

Specifically, we report reductions of approximately 33% in mineral fertilisers and 7% in the total NFUs to obtain the maximum grain yield, which could represent a 67% reduction in mineral fertilisers and a 40% reduction in the total NFUs to obtain the average yield of the area. These findings are in agreement with a recent similar study, where a reduction of between 18 and 54% of mineral fertiliser as a function of organic amendment applied was estimated [60]. Another recent study quantified similar values with a decrease of 22% in the use of mineral fertiliser [94]. Additionally, similarly, sewage sludge (aerated and dewatered) was reported to show the highest potential for mineral fertiliser replacement [60].

The good performance of the CF strategy translates into a higher NAE. Treatments 120+ and 60+ also showed the highest NAE values, which further suggests they are the most efficient alternatives among the studied treatments (Table 3). Generally, the NAE is a function of the N application rate, crop type, and nitrogen fertiliser applied [31]. In our study, an increased NAE when CF was applied could be due to the existence of fewer losses from the system motivated by the reduction in the inorganic, more labile N [29,31,63]. In addition, the organic amendment effect on the regulation of the soil quality, root growth, and the N accumulation postanthesis [62,95–97] likely contributed to increasing the NAE as well. From this perspective, previous studies have similarly shown an increased nitrogen use efficiency when CF was adopted [62,95,97–100].

For a rational use of biosolids, their contaminant content must also be considered, as their use can be related to a possible transmission of heavy metals and contaminants. The biosolid used in this study was fully compliant with the current legislation to be applied on agricultural soil (see Section 2.2 for further details). In addition, a recent study [71] carried out with the same biosolid at a higher application rate and frequency concluded that the concentration of heavy metals in grain and soil after 26 years of application were below the legislative levels. Therefore, the application of this biosolid as an organic amendment in

the edaphic, climate, and agricultural management conditions of our study is compatible with soil health and the promotion of the circular economy.

4.2. Soil Organic Carbon and Soil Total Nitrogen Storage

In our study, *BioSolid* and *Nmin*, but not their interaction, were significant in explaining organic C storage, and the rate of the carbon storage per unit of NFU added was not statistically different, irrespective of whether the biosolid was added or not. The combined organic–inorganic fertilisation showed the highest soil organic carbon (SOC) stock values (Figure 2). No differences in SOC stocks were observed among the mineral-only treatments or among the CF treatments. A significant difference was observed between some CF treatments and the lower *Nmin* mineral-only treatments (*BioSolid*: No; *Nmin*: 60 and 120) and between the CF treatments and the control treatment (*BioSolid*: No; *Nmin*: 0). This suggests that, at low NFUs, the CF is more effective in storing C than the mineral-only fertilisation. Nevertheless, despite the fact that the combined organic–inorganic fertiliser combinations showed the highest SOC stock values (Figure 2), these stocks were not statistically distinguishable from the SOC stock shown by the reference mineral fertilisation (*BioSolid*: No; *Nmin*: 180).

An organic amendment application was also one of the most effective strategies favouring C storage in a recent study conducted in the region [16]. In a recent meta-analysis assessing carbon storage after biosolid application to soil, four variables were found to be related to SOC stocks. The cumulative rate of the biosolid was the main factor affecting soil C content, followed by time after application, soil depth, and the C content of the biosolid [4]. The significance of *Nmin* in increasing SOC is also consistent with previous studies [33,34,36,101]. However, we did not observe a higher SOC accumulation for the CF compared to the reference fertilisation (*BioSolid*: No; *Nmin*: 180). This could be due to the management of the trial, where straw was removed. In this respect, the fertiliser technique used favoured the development of aerial biomass (grain and straw). With the application of mineral fertilisers [32] and a biosolid [47], this vegetative development increased. The biomass increase is suggested to be particularly acute for organic amendments with a C:N ratio of 10 or lower [102], ratios similar to the ones observed for our biosolid (Table 1). Nonetheless, the highest aerial vegetative growth was observed with the use of CF [61,62,103–105]. Leaving straw in the field is a practice usually recommended to increase the SOC stock [11,12,106]. In fact, a study also testing a CF strategy found a significant correlation between wheat biomass and SOC and STN in the 0 to 20 cm soil layer [105]. Therefore, by removing the straw, a large amount of organic C would be lost from the system, preventing further SOC storage potential. On the other hand, the low SOC accumulation with the use of CF may also be related to an increased emission of gases produced by microbial respiration after biosolid application [55,56,107,108]. Organic amendments with a low C:N ratio, such as the one in our study (Table 1), have been found to produce higher CO₂ and N₂O emissions when combined with mineral fertilisation [84,103,109]. Therefore, soil respiration in CF approaches would be favoured by the low C:N ratio of biosolids, which have a high amount of C and labile N available as a consequence of incomplete reactions of anaerobic digestion [107,110].

When it comes to STN, the *BioSolid* factor was found to be the sole factor to significantly increase STN, with a significantly higher stock being observed when it was applied (Figure 3). A simultaneous increase in SOC and STN content as a consequence of the application of organic amendments has been previously documented [36,63,111,112]. This simultaneous improvement would be related to the linear correlation of STN with SOC, especially for the CF treatments [63]. Furthermore, the application of organic amendments is related to an increase in the light and heavy fractions of soil organic matter, contributing to the stabilisation of SOC and STN [51,113]. In this sense, the increase in SNT would contribute to the stable storage of SOC [18,19].

5. Conclusions

Our findings suggest that with the agronomic use of a biosolid, the amount of mineral fertiliser needed to obtain yields similar to the reference treatment (180 mineral NFUs) decreased, even 3 years after the last application of the organic fertiliser. This translated to decreased fertilisation needs. Specifically, mineral N and total NFU needs required to obtain yields similar to the reference mineral-only treatment decreased by 33% and 7%, respectively. When compared to the mean local crop production, the combined fertilisation obtained similar yields with 67% less mineral fertiliser and 40% fewer total NFUs. These results may be related to a higher NAE, which was observed in combined fertilisation treatments whose fertilisation properties did not exceed the total NFUs required by the crop. From this vantage point, this contribution demonstrates that the use of an organic–mineral fertilisation strategy would simultaneously allow for a reduction in expensive mineral fertilisers while maintaining soil organic carbon storage, soil total nitrogen storage, and average regional grain yields. Considering that soil amended with this biosolid over a long period of time has been reported not to exceed the trace metals allowed by the national and European legislation when used at the rates in this study, these combined organic–mineral strategies are well aligned with the simultaneous improvement in soil health and the promotion of the circular economy.

This contribution also suggests the need for assessing the efficacy of each agricultural technique locally to avoid overestimating or underestimating their potential to produce food, while maintaining or improving soil functioning and health.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agriculture13040860/s1>, Table S1. Two-way ANOVA table for grain yield (kg ha^{-1}). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factors Biosolid (2 levels), dose of mineral fertiliser nitrogen (N_{min} , 5 levels), and the interaction between both ($BioSolid:N_{min}$) are presented. Table S2. ANCOVA table for grain yield (kg ha^{-1}). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factor Biosolid (2 levels), the covariate dose of mineral fertiliser nitrogen (N_{min} , 3 levels), and the interaction between both ($BioSolid:N_{min}$) are presented. Table S3. One-way ANOVA table for nitrogen agronomic efficiency ($\text{kg Nitrogen Fertiliser Unit}^{-1}$). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factor fertiliser-treatment (9 levels) are presented. Table S4. Two-way ANOVA table for soil organic carbon (kg ha^{-1}). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factors Biosolid (2 levels), dose of mineral fertiliser nitrogen (N_{min} , 5 levels), and the interaction between both ($BioSolid:N_{min}$) are presented. Table S5. ANCOVA table for soil organic carbon (kg ha^{-1}). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factor Biosolid (2 levels), the covariate dose of mineral fertiliser nitrogen (N_{min} , 5 levels), and the interaction between both ($BioSolid:N_{min}$) are presented. Table S6. Two-way ANOVA table for soil total nitrogen (kg ha^{-1}). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factors Biosolid (2 levels), dose of mineral fertiliser nitrogen (N_{min} , 5 levels), and the interaction between both ($BioSolid:N_{min}$) are presented. Table S7. ANCOVA table for soil total nitrogen (kg ha^{-1}). The Sum of Squares (Sum Sq), Degrees of freedom (Df), F value, and p-value (Pr(>F)) for the factor Biosolid (2 levels), the covariate dose of mineral fertiliser nitrogen (N_{min} , 5 levels), and the interaction between both ($BioSolid:N_{min}$) are presented.

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