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25 years of continuous sewage sludge application vs. mineral fertilizers on a calcareous soil affected pH but not soil carbonates

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Inorganic and organic fertilizers have been widely used to maintain crop yields. However, several studies have demonstrated that the dissolution of carbonates in agricultural soils by the acidification induced by N-fertilizers can result in their total or partial loss in the tilled layer of some agricultural soils. The effect of inorganic and organic fertilization on carbonates in calcareous semiarid Mediterranean soils has been less studied and is still unclear. Based on a 25-year field experiment, we investigated the effects of different fertilization strategies on the soil pH, inorganic C content, and in the proportion of pedogenic carbonates in the topsoil (0–30 cm) of a carbonate-rich soil in Navarre (N Spain). Five treatments were compared: no amendments as a control (SC), mineral fertilization (MF), and the application of sewage sludge at different doses (80 Mg ha⁻¹ every year (80-1), 40 Mg ha⁻¹ every year (40-1) and 40 Mg ha⁻¹ every four years (40-4)). Results showed a decrease in soil pH values by increasing the amount of sewage sludge and a small alteration in the calcite structure particularly in 40-4. However, no significant differences between treatments were found in the total content of carbonates nor in the proportion of pedogenic carbonates. The high concentration of total carbonates in the soil (16.09 ± 0.48%), and of the proportion of pedogenic carbonates (40.21 ± 1.29%, assuming a δ¹³C of primary carbonates = 0 ‰) seemed elevated enough to compensate for the observed acidification in the studied soil. In the case of MF, no changes were observed in the pH values, nor in the carbonate content (total and pedogenic). This suggests that the use of sewage sludge could induce changes in the future at a faster rate and of greater dimension than the use of mineral fertilizers such as the ones used in this field (46% urea, superphosphate and CLK).

KEYWORDS

pedogenic carbonates, soil inorganic carbon, sewage sludge, inorganic fertilizers, semi-arid land

Introduction

Soils are the largest terrestrial C pool and the third greatest C reservoir in the world, following oceans and fossil fuels (1). The soil C pool is formed by organic and inorganic C (SOC and SIC), which cannot be considered completely independent from each other. SOC has been studied in much detail since long ago due to its importance in chemical, biological and physical processes in soils, and playing an essential role in agricultural soil functioning (2). In contrast, the SIC pool (mainly formed by carbonate minerals, and especially, calcite) has not been carefully studied because its long average residence time (millennia), has led to the general assumption that agricultural practices cannot alter the global content or characteristics of SIC at the same pace than those of SOC. However, several studies have shown that agricultural management activities can indeed generate changes in the dynamics of SIC (3–5). These alterations can be reflected in the amounts, mineralogy and morphology of carbonates of carbonates in superficial soil layers in relatively short time lapses (6–9). This is so because agricultural management normally implies changes in the chemistry of the soil solution through for example fertilization, incorporation of amendments such as lime, and irrigation with water of different quality (3, 6, 10–13). Furthermore, agricultural practices change the soil biological activity (i.e. greater or lesser activity of roots and microorganisms), and therefore of the partial pressure of CO₂ (6), which all can alter the SIC cycle. Considering that, calcareous soils account for approximately 50% of the Earth's surface including approximately 9 billion hectares of arable land worldwide (14), the actual relevance of these processes in agricultural soils needs to be quantified for a better understanding of the consequences of agricultural management on the global soil C budget.

This is especially important in arid and semiarid soils developed on calcareous materials, where the largest SIC stocks are commonly found as a consequence of the non-percolating water regime, which hinders the possibility of complete carbonates dissolution and leaching during pedogenesis (1). As such, in many semi-arid Mediterranean soils, a significant proportion of the total soil mass is formed by primary (lithogenic) and secondary (pedogenic) carbonates (15). However, there is a general lack of data on the proportion of both types of carbonates, because it is difficult to analytically distinguish between them, especially when carbonates are found in the fine fraction of the soil, and the parent material is calcareous (16). This represents a major knowledge gap, despite the relevance of total and pedogenic carbonates and their formation pace in several major soil processes. First, carbonates have been regarded as stabilizers of SOC, which can preserve highly humidified organic matter (2). In fact, in semi-arid zones of Spain, calcareous soils have been seen to contain more SOC than neutral to weakly acidic soils under similar management regimes (17). Second, carbonates are

relevant due to their influence in the total C cycle (18). Third, carbonates play a recognized role in stabilizing soil structure (19, 20). Finally, carbonates can have an important influence in soil fertility and agricultural productivity. Therefore, understanding the SIC cycle and the influence of agricultural management (including fertilization) on it in Mediterranean calcareous soils is crucial.

A very relevant management strategy in this sense is fertilization. Nitrogen fertilizers are widely used around the world and their use is expected to increase in the coming decades (21). According to FAO, the total fertilizer nutrient demand was 185,063 million tons in 2016 and this demand was forecast to grow until 200,919 million tons in 2022 (22). The application of inorganic fertilizers produce changes in the chemical and physical properties of agricultural soils. This is so because the use of N-fertilizers can induce soil acidification. Acidification of calcareous soils results in the dissolution of carbonates, increasing CO₂ emissions and the leaching of Ca²⁺. Zamanian et al. (9), have estimated that at least 0.41 Gt C were released irrecoverably as CO₂ to the atmosphere from SIC over the past 50 years due to the application of N fertilization. In the same direction Raza et al. (23) have accounted for 7 million ha of cropland becoming carbonate-free in the last 40 years in China. Another recent example is the article of Tao et al. (24), which has concluded that Chinese croplands have lost 27–38% of their inorganic carbon density from the 0–40 cm soil layer, and that the soil pH has decreased by 0.53 units over the past 30 years. In that study, N fertilization was one of the major drivers leading to dramatic losses soil carbonates, so that five provinces of China have already lost more than 75% of their SIC stocks from the upper 40 cm soil layer during the last four decades (23). In the same way, Ortiz et al. (25) have concluded that irrigation waters and fertilizers load can dissolve SIC.

In addition to the use of inorganic fertilizers, the use of organic amendments and fertilizers such as sewage sludge (SS) can also cause changes in the SIC content. Sewage sludge has been applied for decades as a soil amendment in many areas. From a circular economy perspective, utilizing SS in agriculture closes the product life cycle (26) and therefore can be one of the most economically sustainable disposal methods. Because of this, many researchers have studied the potential of SS as a source of nutrients for crops and as a fertilizer, because SS can have considerable quantities of nutrients (0.5–0.7% phosphorus and 2.4–5.0% nitrogen according to Gherghel et al. (27)). However, SS can also contain organic and inorganic pollutants and pathogens, which present a risk for the environment (28–30). In addition, the application of SS can also induce changes in some chemical and physical properties of the soils (31, 32). For example, a decrease in soil pH values have been reported by many researchers after the application of SS at high doses and/or over relatively long periods of time (31–36). As with the application of inorganic fertilizers, the decrease in pH values by employing SS can cause carbonate dissolution (14).

Organic and inorganic fertilization can therefore change the inorganic carbon cycle of agricultural soils. Thus, taking into account that the presence of carbonates in soils is crucial for various processes such as acidity buffering, aggregates formation, organic matter stabilization, microbial and enzyme activities, nutrient cycling and plant productivity (23), the study of SIC content should be a priority, especially in areas with high organic or inorganic N fertilization.

In this framework, the hypothesis of this work was that the application of SS or inorganic fertilizers in a calcareous agricultural soil for a relatively long period of time changes the soil pH and, as a consequence, also modifies the SIC stock and typology i.e. lithogenic and pedogenic carbonate types. Therefore, this work aimed to determine the extent of changes in the soil pH, and in the SIC pool in terms of quantity and typology in the tilled layer (0-30 cm) of a calcareous Mediterranean soil after the application of SS and inorganic fertilizers for 25 years. In order to confirm this initial hypothesis, the methodological approach consisted in a two-step process:

- i. First, a mineralogical characterization of the soil samples after the application of SS or inorganic fertilizers for 25 years using X Ray Diffraction (XRD), coupled to the quantification of soil pH and SIC.
- ii. Second, a study of the type of carbonates (lithogenic versus pedogenic), from the quantification of pedogenic carbonates using isotopic determinations.

Materials and methods

Site description and soil sampling

The study was carried out in a long-term experimental field in Arazuri, Navarre, Spain. The experimental field was setup in 1992 to assess the effect of the continuous application of SS as an agricultural amendment in a Calcaric Cambisol (37). The soil has a silty-clay-loam texture (31% clay, 30% silt, 39% sand) and around 16% carbonates in the upper layer (0-30 cm). The main physical-chemical characteristics of this soil layer are shown in Table 1 (30, 32).

The experimental design consists of a random factorial block design with three blocks ($n = 3$) where each block includes five experimental plots corresponding to the five treatments studied. These were: (1) application of 40 Mg ha⁻¹ of SS every year (40-1, corresponding to the average applied dose in production fields), (2) application of 40 Mg ha⁻¹ of SS every four years (40-4, the lowest dose studied), (3) application of 80 Mg ha⁻¹ of SS every year (80-1 as the highest dose), (4) application of mineral fertilization at the agronomic rate every year (MF) and (5) control plot (SC, no SS or other fertilizers were applied). The

TABLE 1 Physical and Chemical Properties of the control plot (0-30 cm) (Arazuri, Spain).

Physical and Chemical Properties in the control plot (Arazuri, Spain)

pH	8.67 ± 0.03
Electrical Conductivity (dS m ⁻¹)	0.169 ± 0.01
Bulk density (g cm ⁻³)	1.59 ± 0.08
Carbonates (%)	16.1 ± 0.48
Organic Carbon (%)	1.35 ± 0.02

individual plots per block, corresponding to the different treatments, had a surface area of 35 m² (10 m x 3.5 m).

The crops used corresponded to the most frequent ones in rainfed conditions in the area, in a rotation of 3 years (cereal-cereal-sunflower (*Helianthus annuus* L.)), managed with annual tillage with a 30 cm deep moldboard plow, and application of phytosanitary products according to the crops' needs each year. The cereal crops used were wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Sewage sludge was produced in the urban wastewater treatment plant from the city of Pamplona (population 330,000), with primary and secondary treatments, stabilized through anaerobic digestion and mechanical dewatering (Table 2). The SS was applied in September, 3 to 4 weeks before sowing the cereal crops, using a 3.5 m wide spreader trailer, followed by moldboard plowing down to 30 cm. Finally, mineral fertilization was done with commercial fertilizers at the recommended dose (180 kg N ha⁻¹ year⁻¹ for wheat, 160 kg N ha⁻¹ year⁻¹ for barley and 100 kg N ha⁻¹ year⁻¹ for sunflower). Nowadays, 46% urea and superphosphate are used for wheat and barley and 46% urea, superphosphate and CIK is used for sunflower. However, other types of mineral fertilizers, such as ammonium nitrate, were used in the past.

Soil sampling was carried out 25 years after the onset of the experiment, in September, at each treatment and replicate at 0-30 cm, corresponding to the tilled depth, after the crop cycle was completed, before the application of SS and at the furthest moment in time from previous soil alterations. Samples were collected using

TABLE 2 Chemical Properties of the Sewage sludge from the urban wastewater treatment plant of Pamplona.

Chemical Properties of the Sewage Sludge

pH	8.16 ± 0.03
Electrical Conductivity (dS m ⁻¹)	1.79 ± 0.02
Total N (%)	5.85 ± 0.13
Ammonium-N (%)	0.75 ± 0.02
Phosphorus (%)	2.43 ± 0.008
Potassium (%)	0.51 ± 0.04
Calcium (%)	5.70 ± 0.20
Magnesium (%)	0.66 ± 0.03
C/N	5.35 ± 0.08

an Edelman-type auger ($\varnothing = 5$ cm). Three subsamples were collected per plot and combined to obtain a composite sample. The samples were then air-dried and ground to pass a 2 mm sieve.

Mineralogical characterization and pH value of the samples

Homogenized samples, finely ground by using an agate mortar were analyzed by powder X-ray diffraction (XRD). The mineralogical composition of the samples was resolved on a PANalytical X'Pert PRO X-ray diffractometer (Malvern, Panalytical, United Kingdom) fitted with a Cu anode. The operating conditions were 40 mA, 45 kV, divergence slit of 0.5°, and 0.5 mm reception slits. The samples were scanned with a step size of 0.0167° (2 θ) and 150 ms per step. The characterization of samples was carried out using the random power method operating from 5° to 80° (2 θ) (38).

In addition, bulk samples were analyzed for their total carbonate concentration by a modified Bernard's calcimeter after quantifying the CO₂ produced after treating a soil sample ground to a powdery consistence with HCl 6M (39).

Finally, taking into the account that changes in SIC are affected by soil pH (40), pH values were analyzed in a soil:water suspension at 1:2.5 after overnight shaking in an orbital shaker at 125 rpm, using an electrode CRISON GLP 22 calibrated with standard solutions of pH 4, 7 and 10.

Calculation of the proportion of pedogenic carbonates

Soil samples ground to 2 mm were treated following two different protocols: (1) carbonate removal by acid fumigation for measuring the $\delta^{13}\text{C}$ of SOC (41), and (2) organic matter removal by heating at 550°C during 3 hours for measuring the $\delta^{13}\text{C}$ of SIC.

The isotopic composition of SIC and SOC were measured by Thermo KIEL IV (Thermo Fisher Scientific). The precision is 0.05‰ for $\delta^{13}\text{C}$. This isotopic signature was used to determine the type of carbonates (lithogenic vs. pedogenic). The proportion of pedogenic carbonates (f) can be estimated using the equation proposed by Salomons & Mook (42).

$$f = \frac{\delta^{13}\text{C}_{\text{carbTOT}} - \delta^{13}\text{C}_{\text{carbPRIM}}}{\delta^{13}\text{C}_{\text{carbPEDOGEN}} - \delta^{13}\text{C}_{\text{carbPRIM}}}$$

where $\delta^{13}\text{C}_{\text{carbTOT}}$, $\delta^{13}\text{C}_{\text{carbPRIM}}$ and $\delta^{13}\text{C}_{\text{carbPEDOGEN}}$ are the isotopic signature of total, primary or lithogenic and pedogenic carbonates, respectively.

Such a proportion cannot be directly calculated in bulk soil samples nor for any given fraction, because (i) pedogenic carbonates cannot be physically isolated from primary carbonates, which

implies that data on $\delta^{13}\text{C}_{\text{carbPEDOGEN}}$ were not available, and (ii) $\delta^{13}\text{C}_{\text{carbPRIM}}$ is unknown. The determination of theoretical $\delta^{13}\text{C}_{\text{carbPEDOGEN}}$ values is however possible from the isotopic signature of the original soil CO₂ from which they were precipitated, assuming that the isotopic composition of respired CO₂ is equivalent to that of SOC (43), and that respired CO₂ accounts for most of the soil atmosphere CO₂. According to this approach, the isotopic signature of pedogenic carbonates would be that of the respired CO₂ plus an enrichment factor due to lower diffusion rates of ¹³CO₂ than ¹²CO₂ (+4.4 ‰, 44), as well as a temperature-dependent factor accounting for isotopic discrimination during carbonates precipitation from CO₂. Following Bughio et al. (6), the estimated value of the latter was of +10.43‰ in our site (for an average annual temperature of 15 °C).

Common values of $\delta^{13}\text{C}_{\text{carbPRIM}}$ found in the literature for lithogenic carbonates range between -2 to 2 ‰ (45). Therefore, a sensitivity analysis was performed by calculating the proportion of pedogenic carbonates within this range of $\delta^{13}\text{C}_{\text{carbPRIM}}$, as previously used in other works (46–48).

Statistical analysis

The three different zones locating each block ($n = 3$) that groups the five studied plots of 35 m² (10 m x 3.5 m) were randomly chosen in the experimental field. In each of the three blocks, the five treatments were randomly assigned too. In this way, the statistical model used takes into account that there may be a random effect of the block in addition to the fixed effect of the treatment.

The effect of the different treatments was analyzed for three variables: the concentration of total carbonates, the proportion of pedogenic carbonates (calculated using a $\delta^{13}\text{C}_{\text{carbPRIM}}$ value of 0.0‰), and the pH values. The treatment effect for each variable was analyzed with a linear mixed model (LMM) that includes the random effect of the block, and the fixed effect of the treatment. Since the different values of the proportion of pedogenic carbonates calculated for different $\delta^{13}\text{C}_{\text{carbPRIM}}$ values (-2.0, 2 ‰) are linearly related, the conclusions obtained from the model for treatment effects on f calculated for pedogenic carbonates with $\delta^{13}\text{C}_{\text{carbPRIM}} = 0$ ‰ are also valid for the other values. When the LMM observed significant differences, 95% confidence intervals were used for pairwise comparison of mean values between treatments.

Results

Mineralogical composition and pH values

According to XRD results, the minerals identified in the studied soil were quartz, calcite, Ca/Na feldspar and minerals from the phyllosilicate and mica groups (Figure 1). No

differences were detected overall in the mineralogical composition of the samples from the different treatments (Figure 1). However, a small displacement of the d-spacing calcite peak (3.03 Å) was observed by XRD in the plots in which SS were applied (Figure 2).

No differences were observed in the values of pH between the control treatment without any fertilization (SC), and MF (Figure 3). However, a decrease in the pH of the samples after the application of SS for 25 years was observed (Figure 3). The greatest reduction in pH values occurred for the highest dose of SS applied to the soil. The LMM confirmed that this effect was statistically significant (p -value=0.0005). A *post hoc* (95% family-wise confidence level) analysis showed that the plots without SS

addition (SC and MF) had indeed higher pH values than those treated with SS at high doses (40-1 and 80-1) (Figure 3).

Quantification of total and pedogenic carbonates

Figure 4 shows the results of the concentration of total carbonates, and the proportion of pedogenic carbonates in the studied treatments. In relation to the total concentration of carbonates, the samples presented a mean value of $16.09 \pm 0.48\%$ (mean \pm se(mean)) of carbonates (Figure 4A). No significant differences were found between treatments (p -value > 0.05

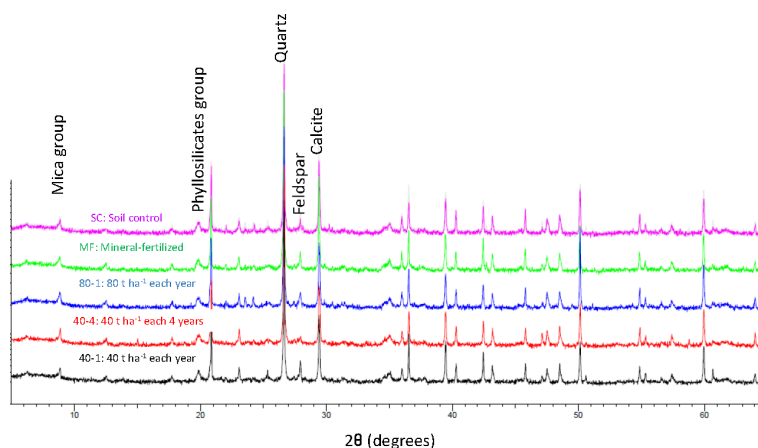


FIGURE 1

Mineralogical composition of the studied plots by XRD. (Application of 40 Mg ha⁻¹ of SS every year (40-1), application of 40 Mg ha⁻¹ of SS each four years (40-4), application of 80 Mg ha⁻¹ of SS every year (80-1), application of mineral fertilization every year (MF) and control plot (SC).

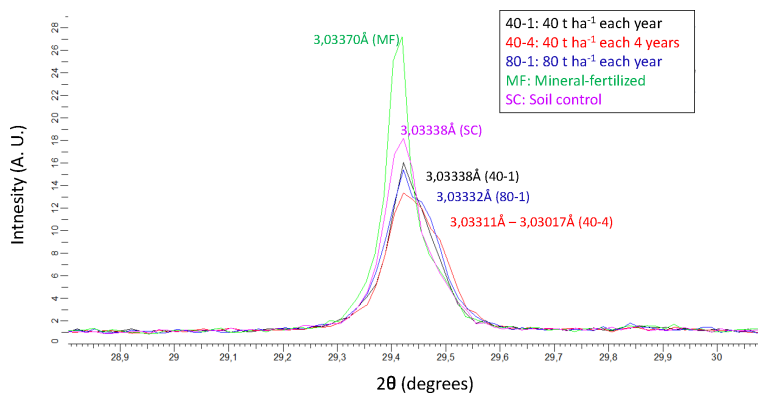


FIGURE 2

Displacement of the calcite peak by XRD (Application of 40 Mg ha⁻¹ of SS every year (40-1), application of 40 Mg ha⁻¹ of SS each four years (40-4), application of 80 Mg ha⁻¹ of SS every year (80-1), application of mineral fertilization every year (MF) and control plot (SC).

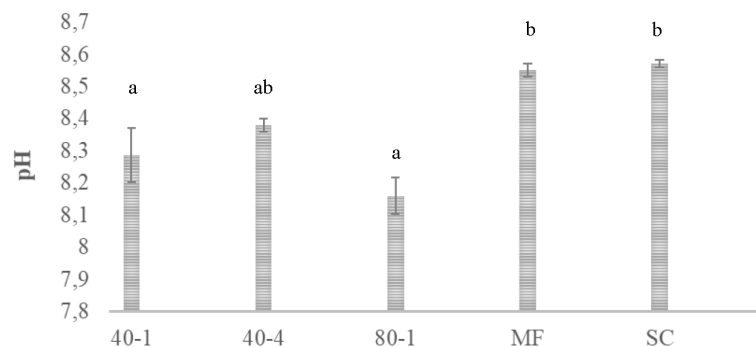


FIGURE 3

Mean pH values \pm se(mean) of the soil samples. (Application of 40 Mg ha⁻¹ of SS every year (40-1), application of 40 Mg ha⁻¹ of SS each four years (40-4), application of 80 Mg ha⁻¹ of SS every year (80-1), application of mineral fertilization every year (MF) and control plot (SC). Different letters mean differences between groups ($p < 0.05$).

corresponding to the fixed effect of the treatment in the considered LMM). Similar statistical results were found for the proportion of pedogenic carbonates f . In this case, the mean value of f was $40.2 \pm 1.29\%$ for $\delta^{13}\text{C}_{\text{carbPRIM}} = 0 \text{ ‰}$ (Figure 4B).

Discussion

Mineralogical composition and pH values

No important changes in soil mineralogy were detected by XRD (Figure 1). Nevertheless, a small displacement of the d-spacing calcite peak (3.03 Å) was observed in the plots in which SS were applied (Figure 2). This small displacement of the d-spacing calcite peak suggests the incorporation of Mg in the calcite structure of pedogenic carbonates (49, 50), indicating that carbonates dissolution/re-precipitation is an active process in SS-treated soils.

This displacement was not appreciated in the MF and SC plots (Figure 2), which means that dolomite or other soluble minerals with Mg were not present in the soil (Figure 1) and the only source of Mg in this experiment is the SS used in the 80-1, 40-1 and 40-4 plots (Table 2) (30). Furthermore, the MF plot showed a narrower calcite peak, suggesting a higher crystallinity than in the other treatments. This fact can be explained because some fertilizers applied in MF plots in the past contained sand-size carbonate grains in their composition (7). Carbonate minerals are intentionally added to some N-fertilizers to reduce their explosive properties and to facilitate their manipulation (51).

In relation to pH values (Figure 3), the decrease in the pH values after the application of SS for 25 years (especially for the highest dose) could be explained by nitrification of the ammonium contained in SS and/or the production of organic acids during organic matter decomposition (52). The relatively high amounts of N added with SS in the treatments with the highest doses can

therefore be related to the observed decrease in pH. It has to be noted that despite this statistically significant differences of pH values, the soil pH remained above 8.0 in all treatments, due to the buffer capacity of the high initial content of carbonates ($16.09 \pm 0.48\%$), as recently summarized by Raza et al. (23).

In contrast, the pH values did not change after 25 years of applying mineral fertilizers (MF). An explanation can be the intentional addition of carbonates in some fertilizers to reduce their explosive properties and to facilitate their manipulation (51). The added carbonates in the MF plot were most probably in fine/loose particles leading to fast reaction, as they were at the place where acidity generated, for example around the fertilizer granules. Another explanation is the rate of acidity generation by organic fertilizers in the treatments using SS. The rate of acidity of SS is relatively slower than that of the mineral fertilizers but the process takes over longer periods, depending on decomposition rates.

When comparing the pH results of the MF plot with data from other parts of the world, an opposite behavior was observed, since a general drop in soil pH values has recently been reported in China after the application of inorganic fertilizers (23, 24). An explanation of this different response of soils is the differences in the type of fertilizer and doses used. There are several types of commercial N-fertilizers such as anhydrous ammonia, aqua ammonia, ammonium nitrate, ammonium sulfate, N solutions, urea, NH_4^+ -phosphates, and mixed fertilizers (53). And the magnitude of the expectable soil pH decrease related to their use is different depending on the type (54, 55) and on the N dose (55). In this study, the average N fertilizer input in MF was $157 \text{ kg N ha}^{-1} \text{ year}^{-1}$, which is smaller than the average N input used in China during 2011–2017 [$240 \text{ kg N ha}^{-1} \text{ year}^{-1}$, even reaching values of $500 \text{ kg N ha}^{-1} \text{ year}^{-1}$ in orchards and greenhouse vegetables crops (23)]. A lesser impact of mineral N fertilization on soil pH in

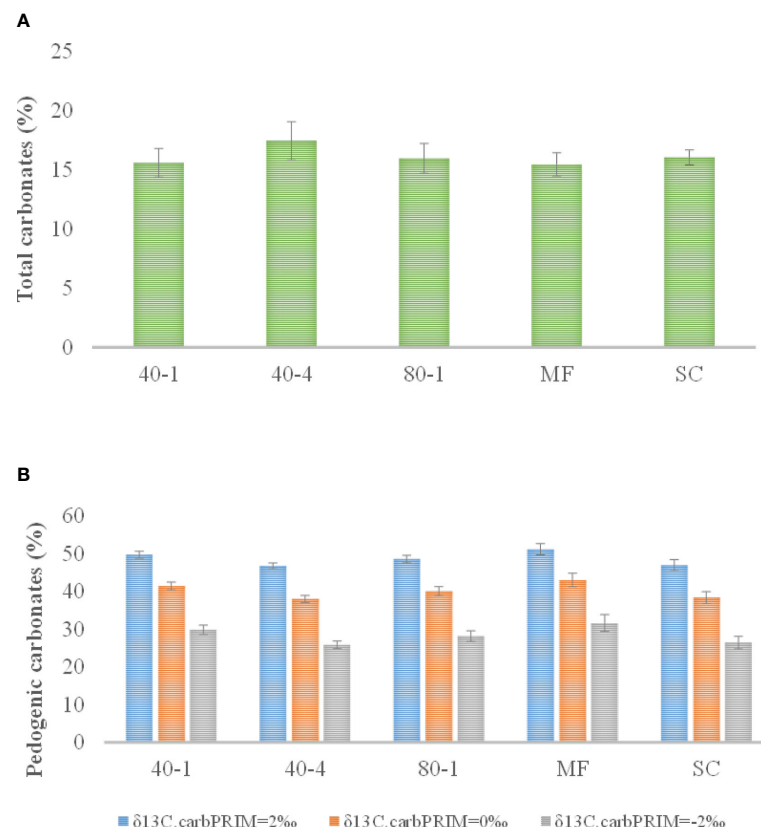


FIGURE 4

Mean values of total content of carbonates \pm se(mean) in the soil samples (0-30cm). (A) concentration of total carbonates. (B): Mean values of proportion of pedogenic carbonates. (Application of 40 Mg ha⁻¹ of SS every year (40-1), application of 40 Mg ha⁻¹ of SS each four years (40-4), application of 80 Mg ha⁻¹ of SS every year (80-1), application of mineral fertilization every year (MF) and control plot (SC).

the studied soil than in other soils receiving higher N doses seems therefore expectable.

In relation to the type of N fertilizer, its acidifying power cannot explain the difference of our results from others reporting pH drops after long-term mineral N fertilization. The potential of H⁺ release by each type of nitrogenous fertilizer is different depending on its composition. For example, (NH₄)₃PO₄ produce the highest amount of H⁺ release, followed by and (NH₄)₂HPO₄ + KCl (NPK) but urea (CONH₂)₂, NH₄NO₃, and NH₄HCO₃ have a smaller acidifying power (23). In the MF plot, commercial fertilizers with different acidifying power have been used during these 25 years like in China where changes in the pH values of the soils have been reported after the use of several nitrogenous fertilizers (23, 24). This means that in this case, the type of N-fertilizer is not a key factor and the most likely explanation for the different behavior observed is the dose used. Also, as explained above, the soil of this study had a high initial carbonate content (16.09 \pm 0.48%), and therefore an important buffer capacity, thus, the high initial content of soil carbonates can counteract to a great extent the increase of possible H⁺ inputs from nitrogenous fertilizers (14). This suggests that the use of

reasonable doses of mineral fertilizers may be enough to mitigate acidification from fertilization in calcareous soils.

Quantification of total and pedogenic carbonates

Unlike expected, no significant differences were found in the concentration of total carbonates between treatments (Figure 4A), despite the reduction in the pH values of the soil (Figure 3) after the application of SS. Although soil acidification due to the application of fertilizers has the potential to accelerate carbonate dissolution rates (3), the high concentration of carbonates in the study soil (16.09 \pm 0.48%), seems elevated enough not to be sensitive to detect these changes in the bulk samples. This suggests that the initial carbonate content is a key factor for observing and detecting these changes in soil bulk samples. Soil carbonates have a well-known buffering capacity, which helps to stabilize pH when an acidic element is added to the soil. Therefore, the changes in the total and pedogenic carbonates content in carbonate-rich soils like the one used in this study seem to be not as rapid as those seen in low-

carbonate soils. This result is consistent with the findings of other researchers. For instance, Wu et al. (56), observed that the decline in topsoil pH in noncalcareous croplands was almost twice that observed in the calcareous cropland soils of a region in China. In another article about soil acidification in China, the results revealed a significant acidification of all topsoils except in the highest-pH soils, which represented only a small percentage of the Chinese cultivated soil (57). In both cases, N fertilizer application was the most important factor inducing soil acidification of croplands.

In relation to the content of pedogenic carbonates, the soil of study also had an important proportion of pedogenic carbonates (Figure 4B). This high content of pedogenic carbonates suggests that the dissolution and re-precipitation of carbonates is indeed a relevant process in the studied soil depth. The relatively low precipitation-to-evapotranspiration ratio in the area would limit the leaching of dissolved carbonates, and can explain this accumulation of pedogenic carbonates at the topsoil, as explained for the *in situ* models for soil carbonates formation and accumulation described by Zamanian et al. (1).

In this sense, as in the case of the total carbonate content, the proportion of pedogenic carbonates (48.68 ± 0.63 to $28.40 \pm 0.82\%$) seemed elevated enough not to be sensitive to detect changes related to the observed decrease in soil pH, when comparing the results of the control plot (SC), with the other treatments (organic and inorganic fertilizers). This observation suggests that the dissolution of carbonates and the acceleration of the formation of pedogenic carbonates in response to the use of acidifying amendments may be modulated by the buffering capacity of the soil associated with its original carbonate content, which seems a crucial factor in this process.

It is also possible that, although no changes in the carbonate content of the bulk samples were detected, changes in some soil size-fractions would occur, as observed in other soils of the region after the adoption of irrigation (7, 47). Therefore, new studies should consider the study of pedogenic carbonates in all the soil size-fractions, considering that other researchers have observed changes in the content of pedogenic carbonates after some agricultural management. For example, Bughio et al. (6), observed annual accumulation rates between 0.23 and 0.12 Mg ha⁻¹ of C in the form of pedogenic carbonates in an irrigated Fluvisol Cambisol in a semi-humid region of China under different fertilization systems (organic and mineral) at medium and low doses, over 39 years.

Conclusions

The use of organic or inorganic N fertilization did not result in carbonate losses in the studied calcareous soil after 25 years, indicating that the changes in this soil component were not as rapid as those seen in low-carbonate soils. This suggests that the buffering capacity of soil carbonates can modulate the dissolution of carbonates and the acceleration of the formation of pedogenic carbonates in response to the use of acidifying amendments. In this

sense, a high soil initial carbonate content could prevent detecting changes in the decadal scale in the total and pedogenic carbonate contents because of acidification. Therefore, the initial soil carbonates concentration seems a crucial factor to this respect. However, a decrease in soil pH values and a slight change in the crystalline structure of calcite in the tilled layer was detected after the application of high doses of sewage sludge for 25 years, but not with mineral fertilization, indicating that the use of sewage sludge could induce changes in the dynamics of soil inorganic carbon in the future at a faster rate and of greater dimension than the use of mineral fertilizers used in this study. The continued use of organic fertilizers should be studied in detail in relation to its acidifying potential in calcareous soils.

Data availability statement

The datasets presented in this article are not readily available because they are subjected to funders' regulations. Requests to access the datasets should be directed to isabelsonsoles.desoto@unavarra.es.

Author contributions

IV and AE were responsible for the conception and design of the study. IDS and IV organized the database and performed formal analysis. HU performing statistical analysis. KZ has done the isotopic analysis. IDS and IV wrote the first draft of the manuscript and AE, HU, and KZ contributed to discussion of the results, review and editing. All authors have read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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