Using Living Plant Systems and Modern Farming Methods to Sequester Soil Organic Carbon, Reduce Greenhouse Gas Emissions and Improve Soil Fertility.

Hassan Sardar[1], Richard Bell[1], David Minkey[2], Luca De Prato[1], Tina Astbury[3], Ravi Parmar[3], Sidharth Kurumangal Sasikumar[3]

[1]Centre for Sustainable Farming Systems, Future Food Institute, Murdoch University, 90 South St, Murdoch WA 6150

[2]WANTFA, 65 Brockway Road Floreat WA 6014

[3]Facey Group, 40 Wogolin Rd, Wickepin, WA 6370

Acknowledgement

We acknowledge the contributions of all the parties and partners who made efforts in different ways to make the experimental work achievable at the Bullaring site, WA (Yealering - Bullaring Rd, Bullaring 6373 WA: - 32°28'37.8"S 117°37'00.3"E). A huge thanks to Luca De Prato who worked hard to establish the trial site including experimental design, probes, loggers and diviner tubes installations and other arrangements. We acknowledge the contribution and generosity of the farmers, Allan and Kelly, who allowed us to utilize their farm for our experiments. We are also grateful to Kaitlyn Anderson (former EO at Facey Group), Tina Astbury (current EO at Facey), Ravi Parmar (Research Coordinator at Facey) and the Facey Group's staff who have been looking after the site and taking measurements and data as required. A big thanks to Wendy Vance from Murdoch University who advised on the devices installed on the site for soil water measurement and guided us how to use those devices and retrieve data from them. We are grateful to the Living Farm staff including Richard Devlin and Orna Tippett who contributed by timely operation on the site for seeding and harvesting etc. And last but not the least, we would like to acknowledge the phenomenal support from the funding organizations, the Department of Primary Industries and Regional Development and the Soil CRC which made this work achievable.

1. Introduction

1.1. Trial Location: Yealering - Bullaring Rd, Bullaring 6373 WA (32°28'37.8"S 117°37'00.3"E)

1.2. Annual Rainfall: The average annual rainfall in the area between 1990 and 2023 is 332 mm (Fig. 2).

1.3. Key messages

- Innovative farming system approaches using plant-based solutions, such as legumes and legumes intercropping, are investigated to increase soil amelioration in terms of biomass, carbon and soil stabilisation and compared to mechanical procedures, including soil disturbance and clay incorporation in deep sandy soils.
- Soil structural disturbance and clay incorporation could improve the reactivity, nutrient retention and weed competition for soil moisture in deep sandy soil.
- Legumes teamed up with soil amelioration practices are an innovative cropping option to improve health of deep sandy soils and increase the soil carbon content versus conventional, soil disturbance and soil clay incorporation.

1.4. Background

Throughout the wheatbelt in Western Australia, there are areas of deep sand with very low fertility, water-holding capacity, non-wetting, compaction, and high acidity. These areas are difficult for growers to manage due to a high risk of erosion, poor crop establishment and, consequently, a lack of biomass and yield.

Innovative farming practices such as soil carbon sequestration, clay amendment and deep ripping are being increasingly recognized as effective strategies for both mitigating the climate change and enhancing soil physio-chemical properties (Godde et al., 2016; Hall et al., 2010; Nazir et al., 2024). For instance, scientific evidence shows that deep ripping, clay amendments and diversified crop rotations enhance soil structure, nutrient availability, and moisture retention (Lal, 2004; Six et al., 2002). Increasing plant species diversity in agricultural systems can improve ecosystem services, including weed and pest suppression, increased pollinator levels, increased land equivalent ratio, and improved yield stability and soil health (Bybee-Finley and Ryan, 2018; Isbell et al., 2017). Increased plant diversity in cropping systems can be achieved through crop rotation or using a pasture phase between cropping cycles where the farming system includes a livestock component. Some form of crop rotation is used in most cropping systems across the world to provide a disease break (Angus et al., 2015), to improve weed control (Sharma et al., 2021), or to diversify income streams to minimise risk (Roesch-McNally et al., 2018). However, many crop sequences in semi-arid areas still focus on cereal crops with greater yield stability, with smaller percentages of oilseeds and pulses in the rotation (Harries et al., 2020). Other management options may therefore be required further to increase the diversity of plants in semi-arid cropping systems. These options include using cover crops, particularly mixed-species cover crops, intercrops or temporary intercrops, or green manure crops.

Therefore, the experiment at Bullaring was focused on integrating living plant systems with modern farming techniques, deep ripping and clay amelioration to improve soil organic carbon, water use efficiency, and overall soil health. The experiment evaluated these innovative approaches in 2022-2024 providing a scientific basis for sustainable management practices that can benefit both the environment and crop productivity. While most amendment or amelioration methods have been evaluated in isolation, this experiment evaluated the stacking of many combinations of amendments and amelioration methods to try and address multiple soil constraints. The hypothesis was that the synergistic benefits of multiple amendments would lead to a long-term increase in soil health and crop grain yield.

2. Methodology

A long-term site to investigate legume soil health amelioration on deep sandy soils was established in the 2021 season. Perennial (lebeckia, lucerne and saltbush) mixed farming systems were sown in a complete block randomised trial and compared to conventional farming systems. Unforeseen lack or poor germination together with spring drought decimated the plants which germinated during the following weeks. Therefore, for the 2022 season, the trial was redesigned to include different farming system approaches, such as soil disturbance, clay amelioration, mixed cropping with legume inter-row sown and an annual legume (Serradella Fran2o).

The trial was re-established in 2022 (sowing date 6th June 2022) as a randomised complete block design with seven crop treatments, including both monocrops and intercrops and four replicated plots per treatment with a base crop rotation (Barley/Lupin/Oat). Total of 28 plots were prepared with 7 plots in each block (Fig. 1). Plot size was 192 m^2 (30 m × 6.4 m). Treatments set used in different years was:

2022

1. Control – Main crop (Barley)
2. Soil disturbance (Bednar) + main crop (Barley)
3. Soil clay incorporation (clay @ 100 t/ha) + main crop (Barley)
4. Lebeckia then later Serradella cv. Fran 2o because the Lebeckia failed to emerge
5. Innovative cropping options sequence 1 - mixed cropping (Oat + vetch)
6. Innovative cropping options sequence 2 - mixed cropping (Lupin + vetch)
7. Innovative cropping options sequence 3 - mixed cropping (Barley + vetch)

2023

1. Control – Main crop (Lupin cv. Jurien)
2. Soil disturbance (Bednar) + main crop (Lupin cv. Jurien)
3. Soil clay incorporation (clay @ 100 t/ha) + main crop (Lupin cv. Jurien)
4. Legume (Serradella cv. Fran 2o)
5. Crop sequence 1 - mixed cropping (Barley cv. Cyclops + Vetch cv. Volga)
6. Crop sequence 2 - mixed cropping (Oat cv. Winjardie + Vetch cv. Volga)
7. Crop sequence 3 - mixed cropping (Lupin cv. Jurien + Vetch cv. Volga)

2024

1. Control – main crop (Oat cv. Bannister)
2. Soil disturbance (Bednar) + main crop (Oat cv. Bannister)
3. Soil clay incorporation (clay @ 100 t/ha) + main crop (Oat cv. Bannister)
4. Legume (Serradella cv. Fran 2o)
5. Crop sequence 1 - mixed cropping (Lupin cv. Jurien + Vetch cv. Volga)
6. Crop sequence 2 - mixed cropping (Barley cv. Spartacus + Vetch cv. Volga)
7. Crop sequence 3 - mixed cropping (Oat cv. Bannister + Vetch cv. Volga)

Clay was incorporated through speed tiller at 20 cm depth. Experimental design used each year is given in Fig. 1 below.

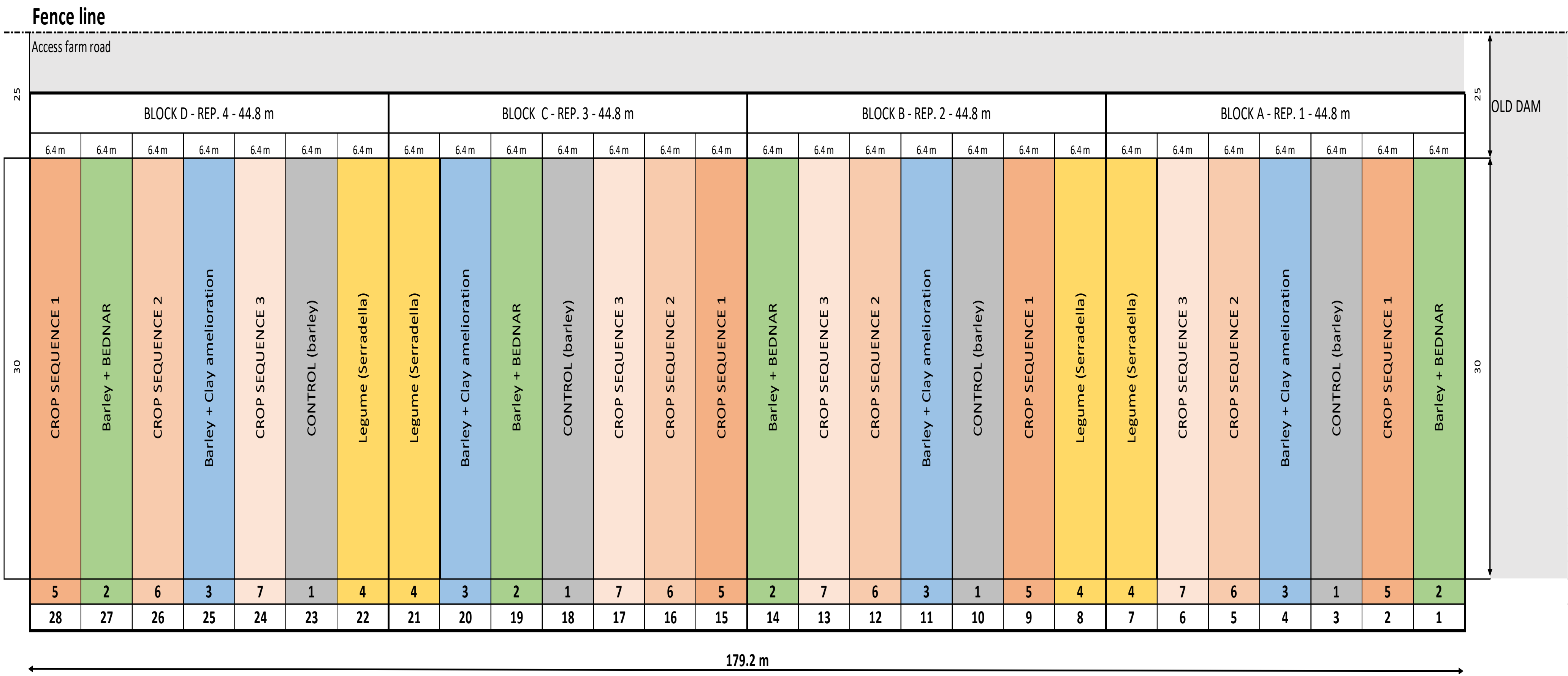


Fig. 1: Experimental design used for the experiment at Bullaring, WA.

2.1. Inputs used in 2022

The trial was sowed on 6th June 2022 with a 2.03 m eight tines trial cone seeder with pressed wheels by a contractor (Living Farm Pty, York, WA). A compound fertiliser MacroPro Extra (N, 10%; P, 11%; K, 11%; S, 10%) was applied at the rate of 60 kg/ha) at seeding. The plot area was previously sprayed for knockdown prior to seeding operations.

2.2. Inputs used in 2023

The plot area was sprayed for knockdown on 20th April (prior to seeding operations) by the local contractor (Living Farm Pty, York, WA). The trial was sowed on 21st April 2023 with a 2.03 m eight tines trial cone seeder with pressed wheels by a local contractor (Living Farm Pty, York, WA). Seed rates for Serradella, Lupin, Oat, Vetch and Barley was respectively 2.00 kg/ha, 100.00 kg/ha, 80.00 kg/ha, 30.0 kg/ha, 70.0 kg/ha. A compound fertiliser (MacroPro Extra at 60 kg/ha) with a composition of 10% N, 11% P, 11% K; and 10% S was applied at seeding. Sulphate of potash was top dressed on 18th August 2023 at the rate of 50 kg/ha. Details of chemicals applied are:

Knockdown/bug sprays: 2 L/ha DST, 1 L/ha chlorpyrifos, 500 mL/ha bifenthrin.

Pre-emergence herbicides applied to treatments 1,2 and 3 (Lupins only): 100 g/ha Metribuzin, 550 g/ha Simazine, 1 L/ha Propyzamide.

2.3. Inputs used in 2024

The plot area was sprayed for knockdown on 20th April (prior to seeding operations) by the local contractor (Living Farm Pty, York, WA). The trial was seeded on 14th May 2024 with a 2.03 m eight tines trial cone seeder with pressed wheels by a local contractor (Living Farm Pty, York, WA). Seed rates for Oat, Lupin, Barley and Vetch was respectively 100 kg/ha, 100 kg/ha, 70 kg/ha, 30 kg/ha. Serradella was not seeded in 2024 as the seed bank from the previous year had sufficiently been regenerated. At seeding on 14th May 2024, CSBP's MacroPro Extra (N, 9.7%; P, 11.2%; K, 11.2%; S, 10.2%; Cu, 0.1%; Zn, 0.2%) was applied @ 120 kg/ha along with the application of Urea @ 25 kg/ha. Fertilisers at seeding were applied using the seeding machine. On 26th June 2024 (6 weeks after sowing), muriate of potash (49.5% K) was applied

@ 40 kg/ha while urea (46% N) was applied @ 60 kg/ha. Details of the chemicals applied in 2024 are given in Table 1 below.

Table. 1. Herbicides/pesticides/insecticides applied at seeding in 2024 (14th May 2024).

Name	Rate	Treatment	Date
Glyphosate	2 L/ha	all except Serradella	14th May
Treflan	1.5 L/ha	Oats only	14th May
Voraxor	200 ml/ha	Oats only	14th May
Chlorpyrifos	800 ml/ha	all except Serradella	14th May
Telstar	100 ml/ha	all except Serradella	14th May

2.4. Plant assessments

To measure plant establishment counts, the number of plants emerged per meter of crop row counted by randomly placing a 0.5m ruler between two rows and counting the number of plants on each adjacent row. Three counts were taken in each plot using the same procedure, then converted to an average number of plants/m^2.

To count the number of weeds/m^2, a pasture square (e.g., 30x30cm) measuring quadrant was randomly placed in the crop and total number of weeds within the quadrant was counted. Three counts were recorded in each plot and converted to average number of weeds/m^2.

NDVI of the crop in each plot was measured using GreenSeeker NDVI meter at three different points within each plot.

Crop biomass of was recorded in the three selected one-meter lines in each plot, then averaged and converted to t/ha.

Grain yield was collected with a trial harvester, and grain yield was recorded in t/ha. grain quality was analysed for protein at CBH laboratory (Perth, WA).

Crop was harvested by the Local contractor, Living Farm, in November-2022, on 1st December in 2023 and on 15th November in 2024.

2.5. Soil analysis

A soil sampling program was developed for full comprehensive nutritional analysis and soil carbon data collection. Soil sampling was carried out at multiple depths (0-10; 10-30; 30-45 cm) to identify soil particle structure and the nutritional constraints for the trial site. Soil sampling methodology included ute-based coring using with bulked samples using 3-4 sampling points in each plot. Each year before seeding, soil sampling was done with analysis conducted by CSBP laboratory (Bibra Lake, WA) on a full comprehensive nutritional package + PBI and Total Soil Organic C measurements.

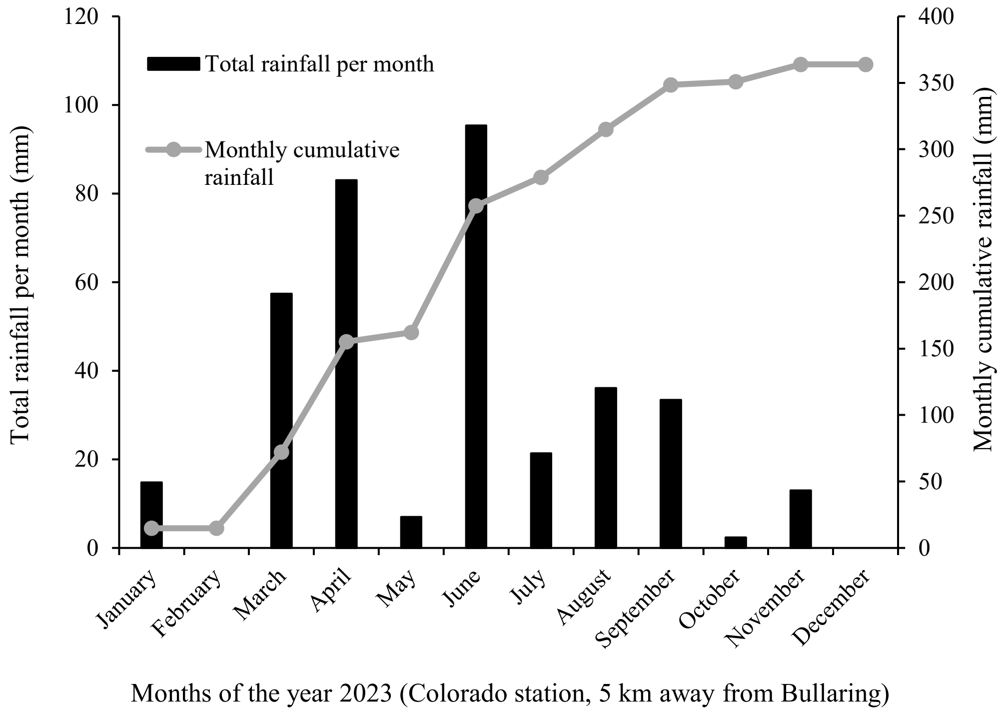
In 2022, samples were collected only from the control plots to get a baseline soil data by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). In 2023, all the treatments were sampled except Serradella. In 2024 and 2025, samples were collected from all the treatments including Serradella.

2.6. Rainfall data (2022)

Total rainfall received in 2022 was 391 mm with greater rainfall received during May-August period and a decline in the monthly rainfall from September onwards (Fig. 3A). May, June, July and August were recorded with 67, 53, 57 and 89 mm rainfall respectively (Fig. 3A).

2.7. Rainfall data (2023)

Total rainfall received in 2022 was 364 mm with greater rainfall received during March (57 mm), April (83 mm) and June (95 mm) (Fig. 3B). A decline in the monthly rainfall occurred after June and little monthly rainfall was received from mid to end of the growing season (July-November) (Fig. 3B).



2.8. Rainfall data (2024)

2024 rainfall data retrieved from the BoM's Colorado weather station (10534) which is 5 km away from the experimental site show that total rainfall received in the first 45 weeks of 2024 is 345 mm (Fig. 3C). A 12.2 mm rainfall was received in the first three weeks of 2024 followed by a 4-weeks dry period followed by a wet period starting from the last week of February and ending in the 3rd week of March (Fig. 3C). An extended dry period of 6-weeks was recorded from the 2nd half of February till the end of April 2024 (Fig. 3C). A 20.6 mm rainfall was received in the first two weeks of May, i.e. before seeding, most of which was received in the first week of May. Seeding on 14th May (start of 20th week of the year) was followed by a dry period of more than a week then followed by a wet period (Fig. 3C). 256 mm of the total rainfall was received during the growing season, i.e. from seeding to harvest (Fig. 3C). The crop received 23 mm and 20 mm rainfall in the 2nd and 3rd week after seeding (22nd and 23rd week of the year), followed by 5 weeks of low rainfall (≤ 10 mm per week). In the 9th and 10th week after seeding, the crop received 26 mm per week (Fig. 3) followed by 3 weeks of medium rainfall (≤ 17 mm per week). 14th week after seeding was again recorded with ample rainfall (23 mm) with a very little rainfall in the following week (Fig. 3C). 36th week of the year (16th week of the crop) was recorded with 18 mm, followed by two weeks with no rain and finally a 6 mm rain in the last week of September-2024 (Fig. 3C). October was dry with only 10 mm rainfall recorded; however, a 15 mm rainfall was received in the 2nd week of November 2024 (Fig. 3C).

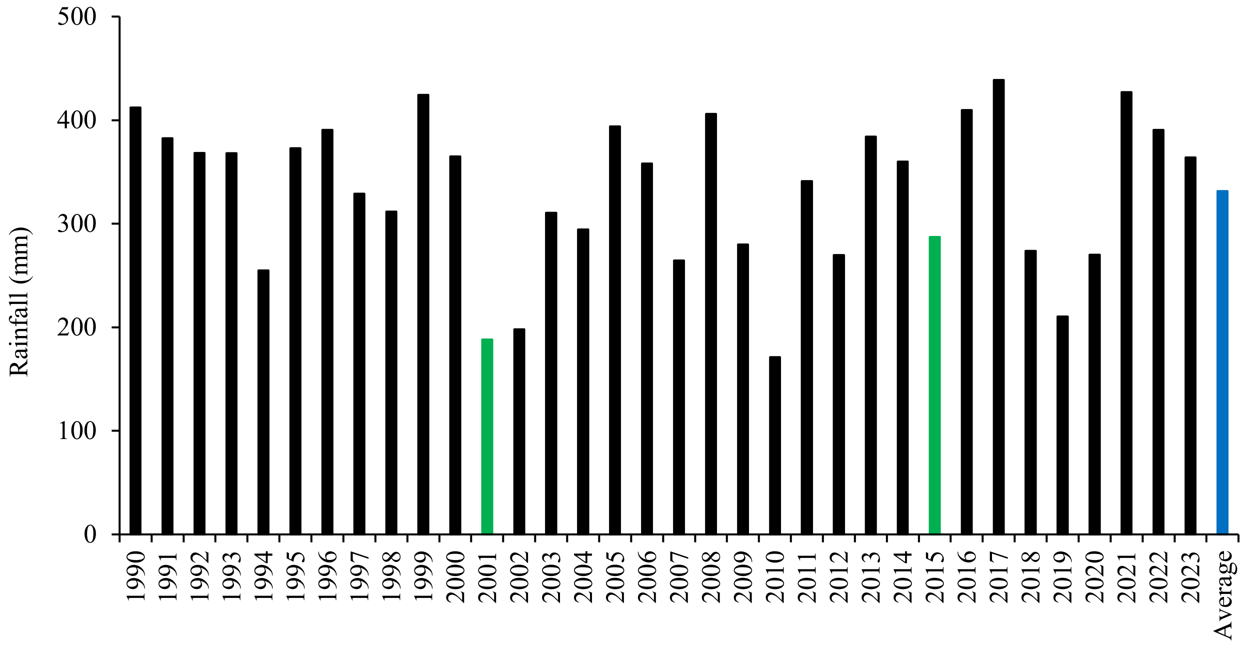
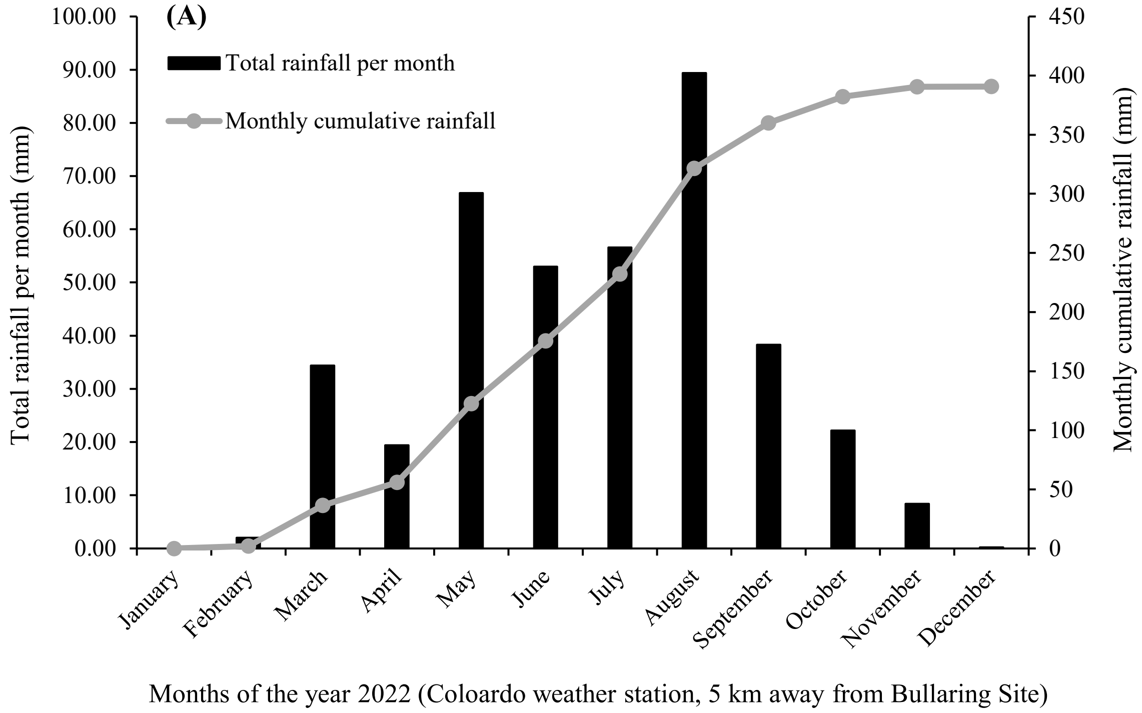
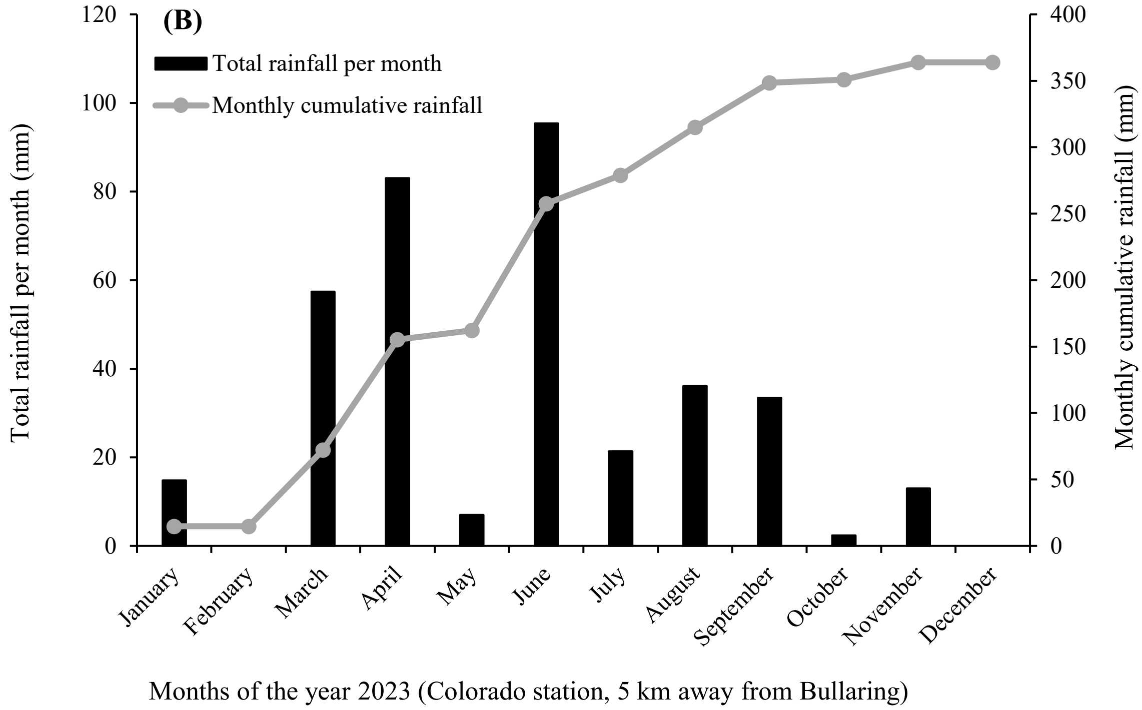


Fig. 2. Total rainfall per year and average rainfall (1990-2023) retrieved from various weather stations of the Bureau of Meteorology of Australia located around Bullaring experimental site. Black bars showing data from the Colorado weather station (10534) which is 5 km away from the site. Green bars showing data from the East Yealering weather station (10912) which is 12 km away from the site. Data from the East Yealering weather station was used where data from Colorado station was not available. Blue bar shows average annual rainfall for 1990-2023 period.


(A)
Total rainfall per month
Monthly cumulative rainfall
Total rainfall per month (mm)
Monthly cumulative rainfall (mm)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
450
400
350
300
250
200
150
100
50
0
January
February
March
April
May
June
July
August
September
October
November
December
Months of the year 2022 (Coloardo weather station, 5 km away from Bullaring Site)


(B)
Total rainfall per month
Monthly cumulative rainfall
Total rainfall per month (mm)
Monthly cumulative rainfall (mm)
120
100
80
60
40
20
0
400
350
300
250
200
150
100
50
0
January
February
March
April
May
June
July
August
September
October
November
December
Months of the year 2023 (Colorado station, 5 km away from Bullaring)

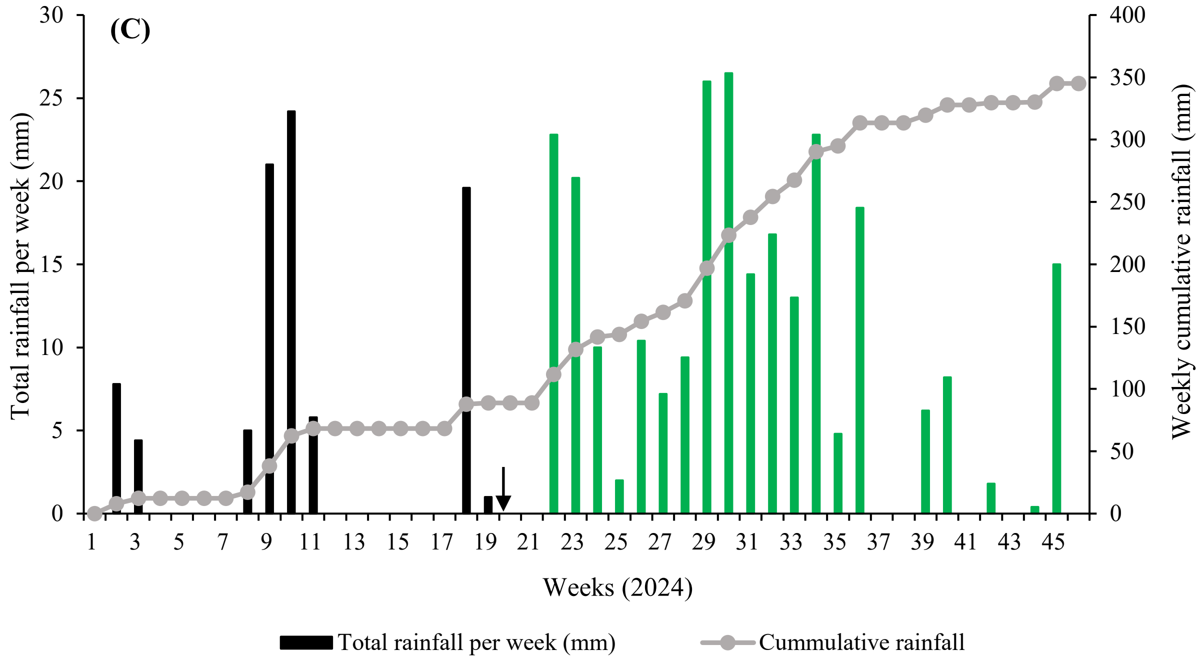


Fig. 3. Rainfall data for **(A)** 2022, **(B)** 2023 and **(C)** 2024 retrieved from the Colorado weather station (10534) of the Bureau of Meteorology of Australia, located at latitude of -32.4825 and longitude of 117.5647. Grey line in panel **(A)** and **(B)** shows monthly cumulative rainfall over time. Black bars in panel **(A)** and **(B)** show total rainfall per month. Black bars in panel **(C)** show total rainfall per week before the seeding was done. Green bars in panel **(C)** show total rainfall per week after the seeding was done. Grey line in panel **(C)** shows weekly cumulative rainfall over time. The arrow in panel **(C)** shows time of seeding.

3. Results and Discussion

3.1. 2022 Crop Results

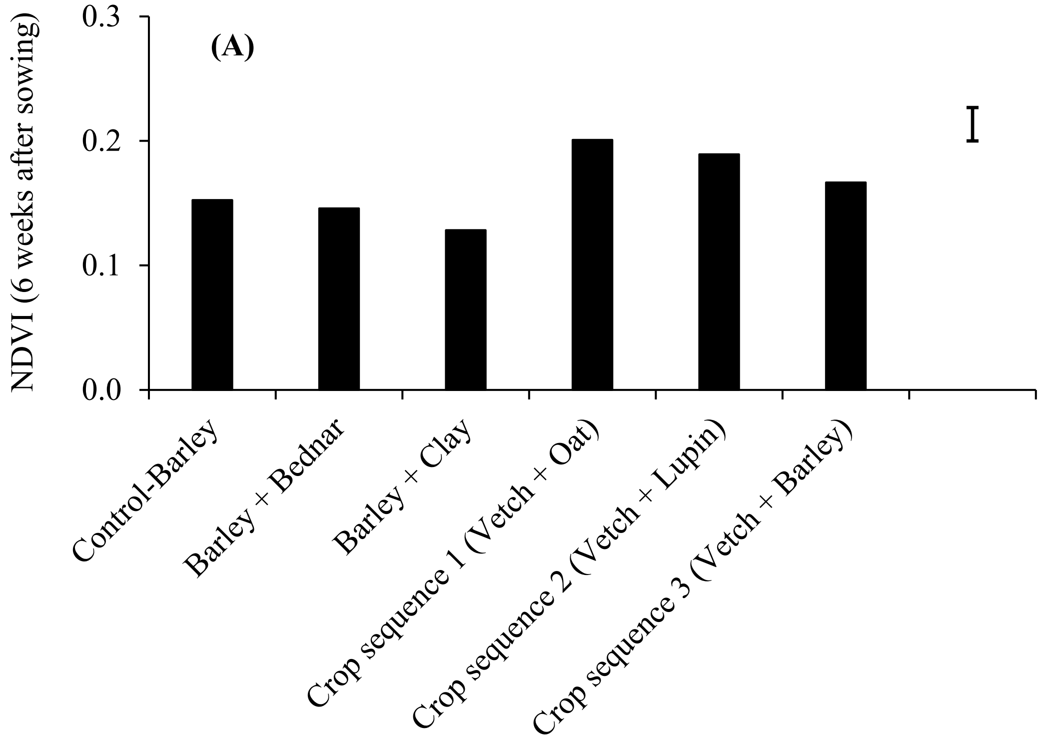
The long-term trial established at Bullaring was focused on assessing the effect of a combination of soil disturbance, amelioration and intercropping practices. No results were collected for plant establishment. In terms of plant health and early biomass, the NDVI (Fig. 4A) showed that the intercropping options had larger value, with the crop mix (oat + vetch) being significantly greater from the barley control and the barley soil amelioration (Bednar and clay amelioration). However, overall, the NDVI scores range between 0.13 to 0.20 (Fig. 4A). Increased NDVI values for the crop mix underlined the early soil cover produced by the intercropping treatments (crop mixes).

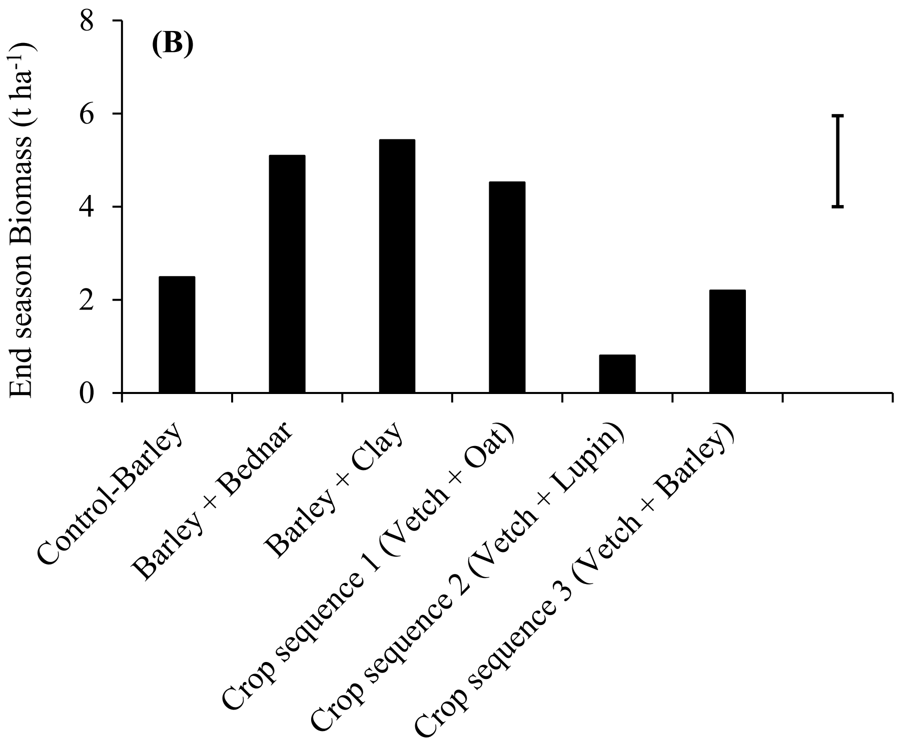
Dry weight at GS80 (Fig. 4B) showed a significant increase of biomass for barley grown under the clay treatment compared to control. Amongst the crop mix treatments, the oat+vetch mix showed the highest, and the lupin mix had the lowest total biomass (Fig. 4B).

The clay-treated plots as well as the Bednar treated plots were found with a significantly greater grain yield relative to control, however, clay was more effective in terms of grain yield relative to the Bednar treatment (Fig. 4C).

The 2022 season of the Bullaring experiment revealed that potential plant productivity improvements could be achieved on unfertile sands using legumes and soil ameliorations approaches.

Intercropping showed increased early biomass and soil cover, as shown by the minor increase in NDVI. However, biomass at GS80 found that mechanical soil ameliorations reported higher dry weight than control and intercropping for barley, probably due to reduced competition for moisture with companion crop (vetch) and/or weeds.





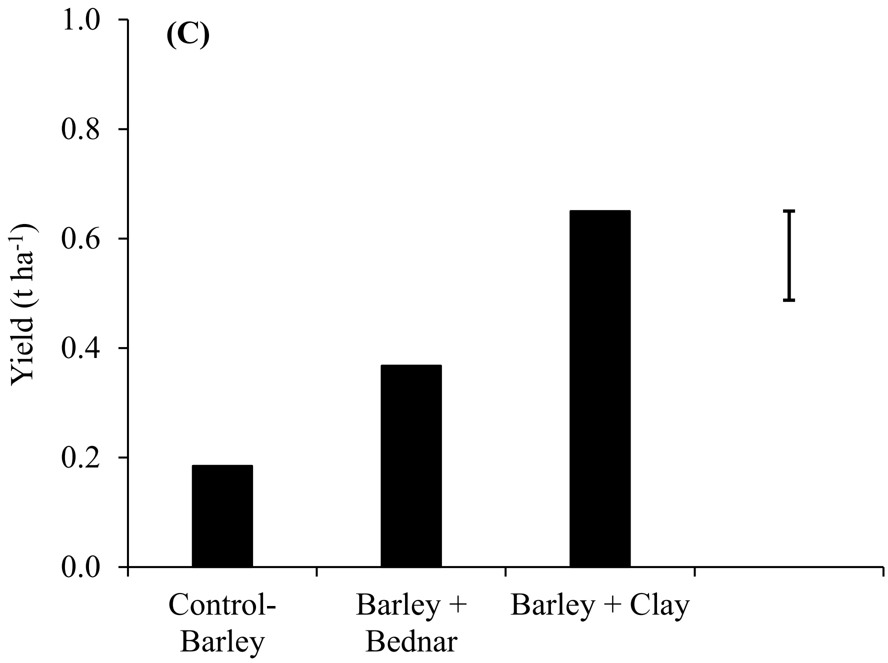


Fig. 4. Effect of different soil and plant-based treatments on **(A)** NDVI, **(B)** End of season biomass at, **(F)** Biomass at the end of season, and **(C)** yield in 2022. Error bar is the LSD value for comparison of means at 95% level of confidence.

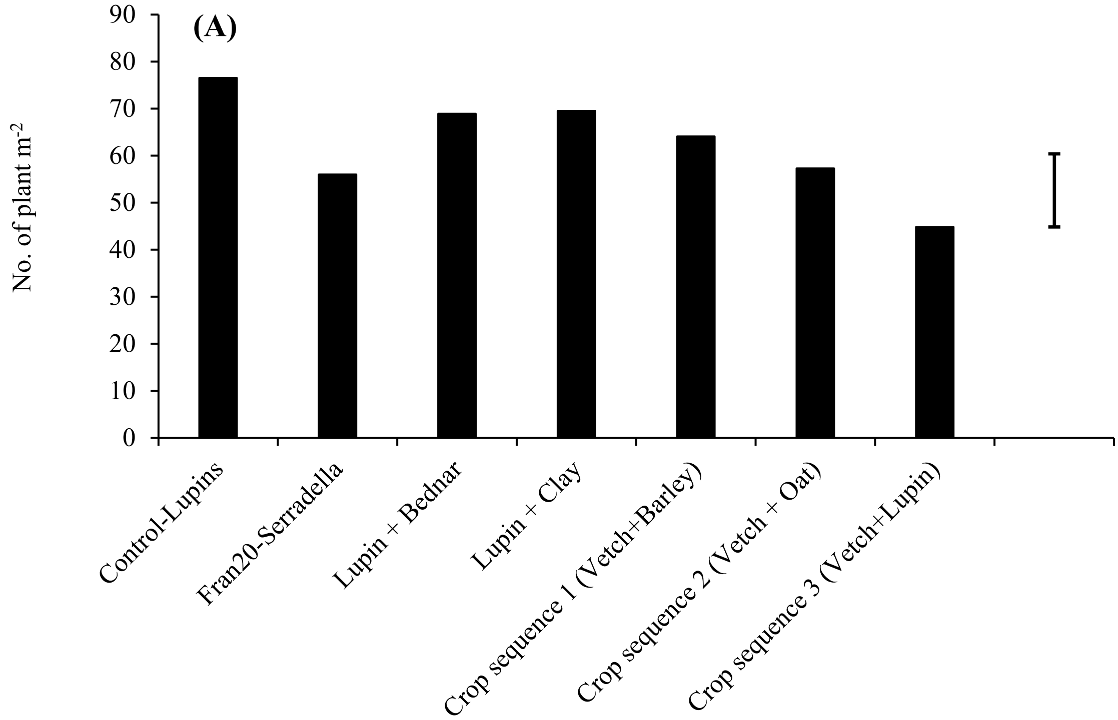
3.2. 2023 Crop Results

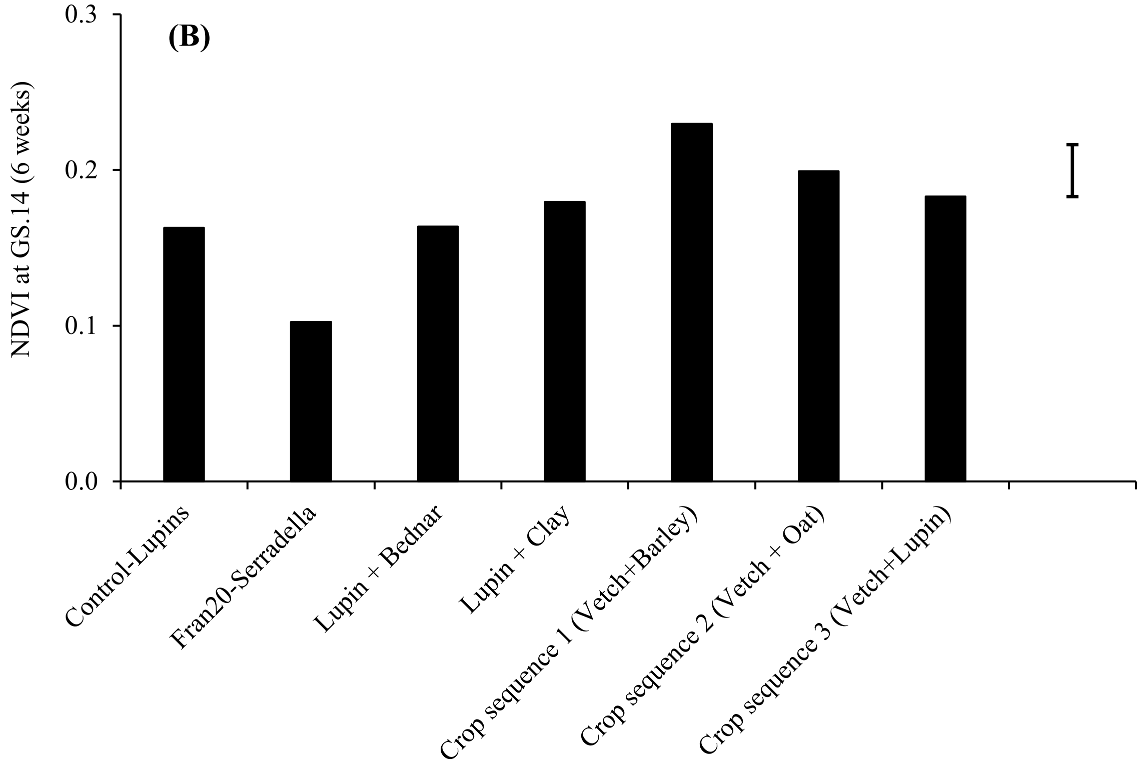
Plants establishment was significantly different between the studied treatments (Fig. 5). Lupins sown solely on all the three soil treatments (control, bednar, and clay amelioration) had similar number of plants (77, 69, 70 plants m^{-2} respectively). Serradella produced 56 plants m^{-2} which is smaller than control. The lowest number of plants m^{-2} (45) was recorded in crop sequence 3 (Vetch + Lupin) which was statistically similar to crop sequence 2 (Vetch + Oat) and Serradella treatments (Fig. 5A).

NDVI of the crop was significantly influenced by the studied treatments at both the stages (GS. 11 and GS. 30). Vetch sown with Barley (Crop sequence 1) was found with the greatest NDVI value of 0.23 NDVI at GS.14 which was significantly greater than all the other studied treatments (Fig. 5B). Serradella had the least NDVI (0.1) at GS.14 (Fig. 5B). Lupin sown on undisturbed soil (Control) had NDVI of 0.16 at GS.14 which was similar to the NDVI of Lupin either sown on Bednar treated soil or clay ameliorated soil (Fig. 5B). Crop sequence 2 and 3 were statistically not different in terms of NDVI at GS.14.

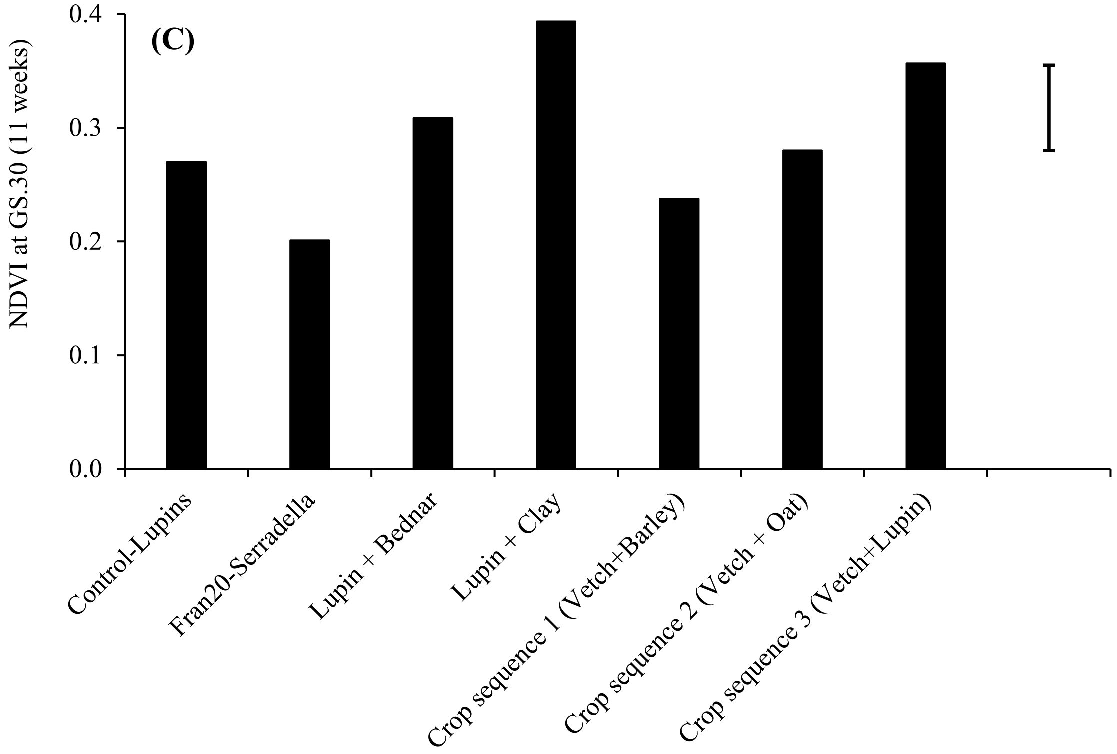
After GS.14, crops under different treatments developed NDVI with different rates over time which altered the difference in their NDVI values at GS.30 relative to the differences between them at GS. 14 (Fig. 5C & D). Lupin sown on clay ameliorated soil enhanced its NDVI magnificently by 0.21 from GS.11 to GS.30 (Fig. 5C & D), thus having the greatest NDVI of 0.39 at GS.30 (Fig. 5C) which is similar to that of crop sequence 3 (Vetch + Lupins) recorded with a 2-fold increase from GS.14 to GS.30 (Fig. 5C & D). On the other hand, NDVI of the crop sequence 1 (Vetch + Barley) remained stagnant between the two growth stages therefore was among the least NDVI producing treatments at GS. 30 (Fig. 5C & D). Serradella also improved after GS.11 but not enough to stand out at GS.30. Clay amelioration and deep tillage have previously been shown to increase crop growth performance by increasing the nutrients availability and improved soil physio-chemical properties (Hall et al., 2015; Hall et al., 2020).

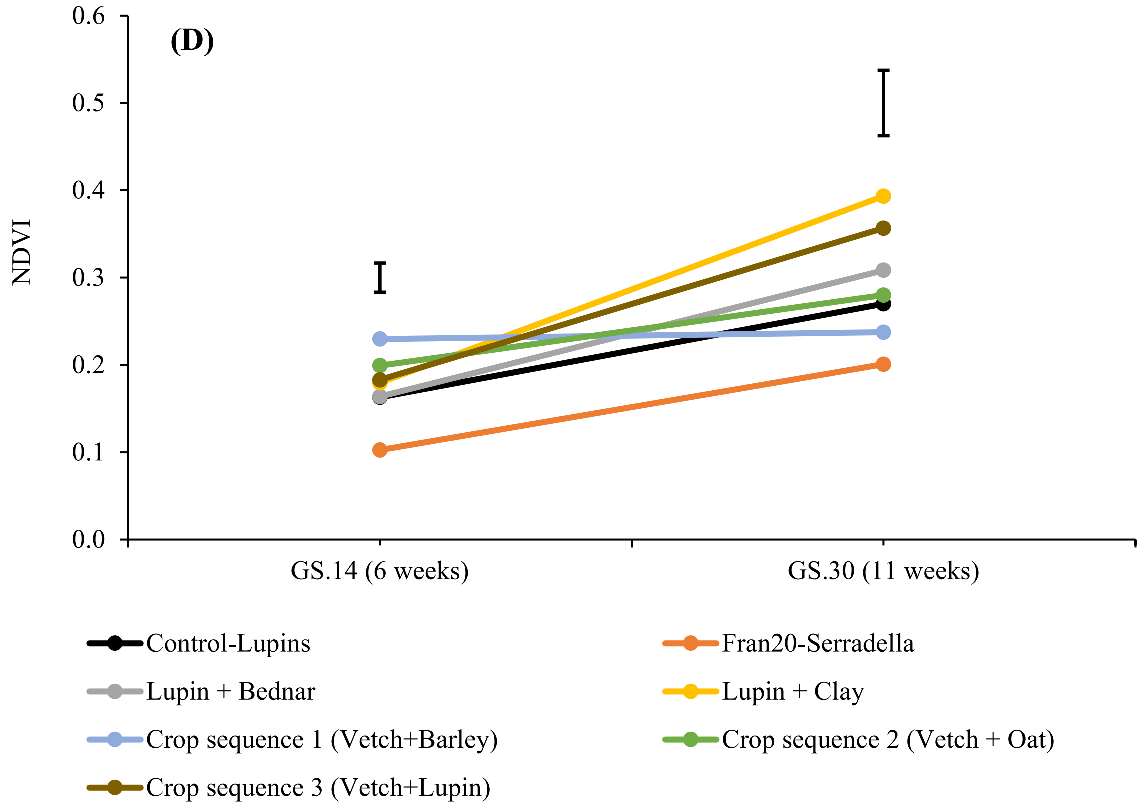
Biomass of the crop under different treatments was not significantly different at GS. 30 but was found significantly different between the treatments at the end of the growing season (Fig. 5E-G). At the end of the season, Lupin crop had similar biomass whether sown on undisturbed soil (7.7 t ha^{-1}), soil inverted with the bednar (10.2 t ha^{-1}) or clay ameliorated soil (8.5 t ha^{-1}) (Fig. 5E-G). This is consistent with the yield data showing no differences between the yield of lupins in any of the mentioned treatments (Fig. 5H). Among the crop sequences, Lupin sown with vetch produced greater biomass (6.51 t ha^{-1}) relative to the other two (Fig. 5E-G).

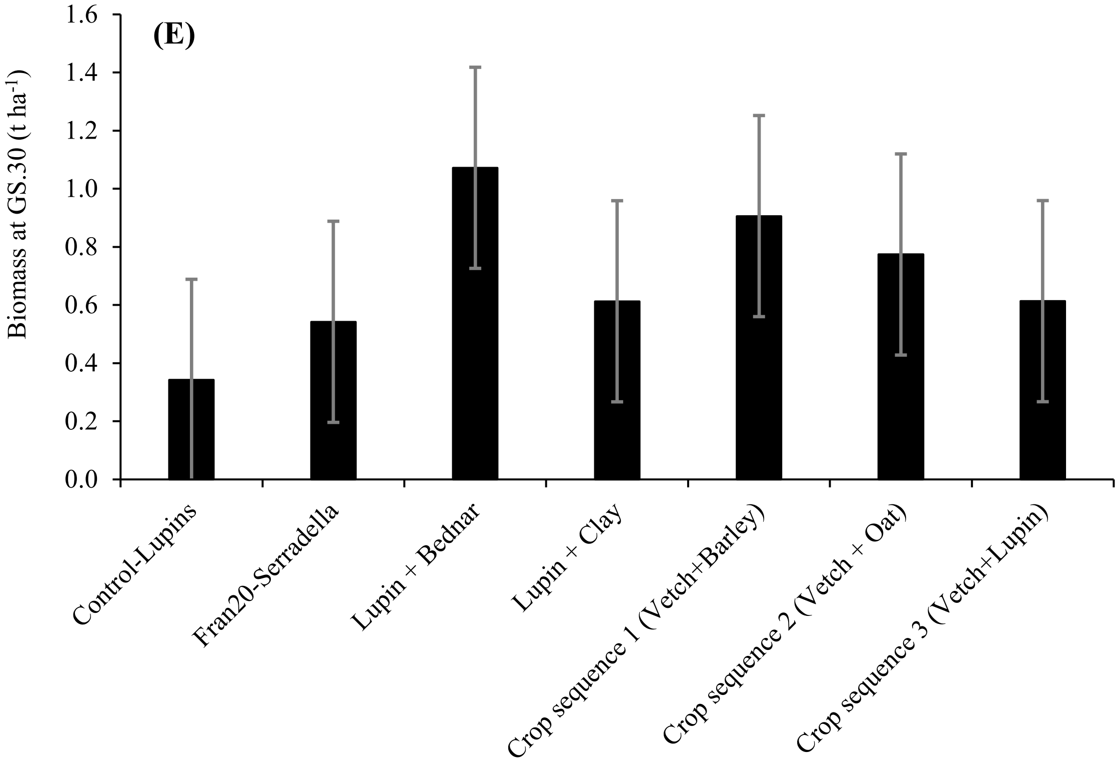


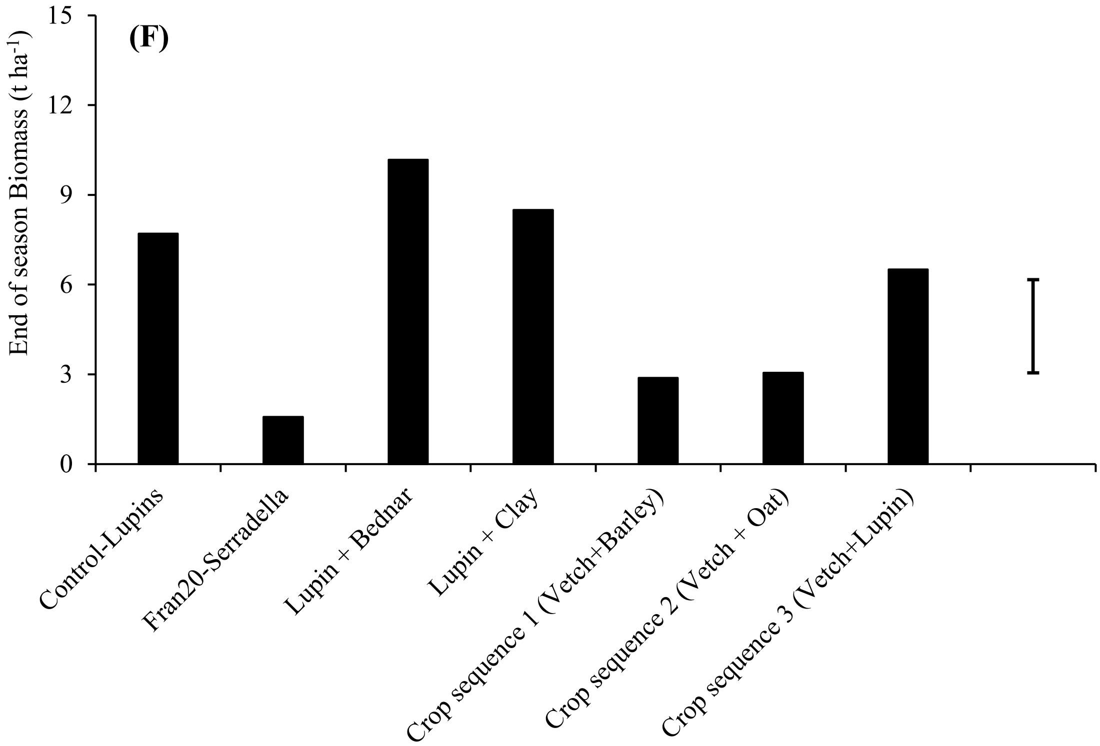


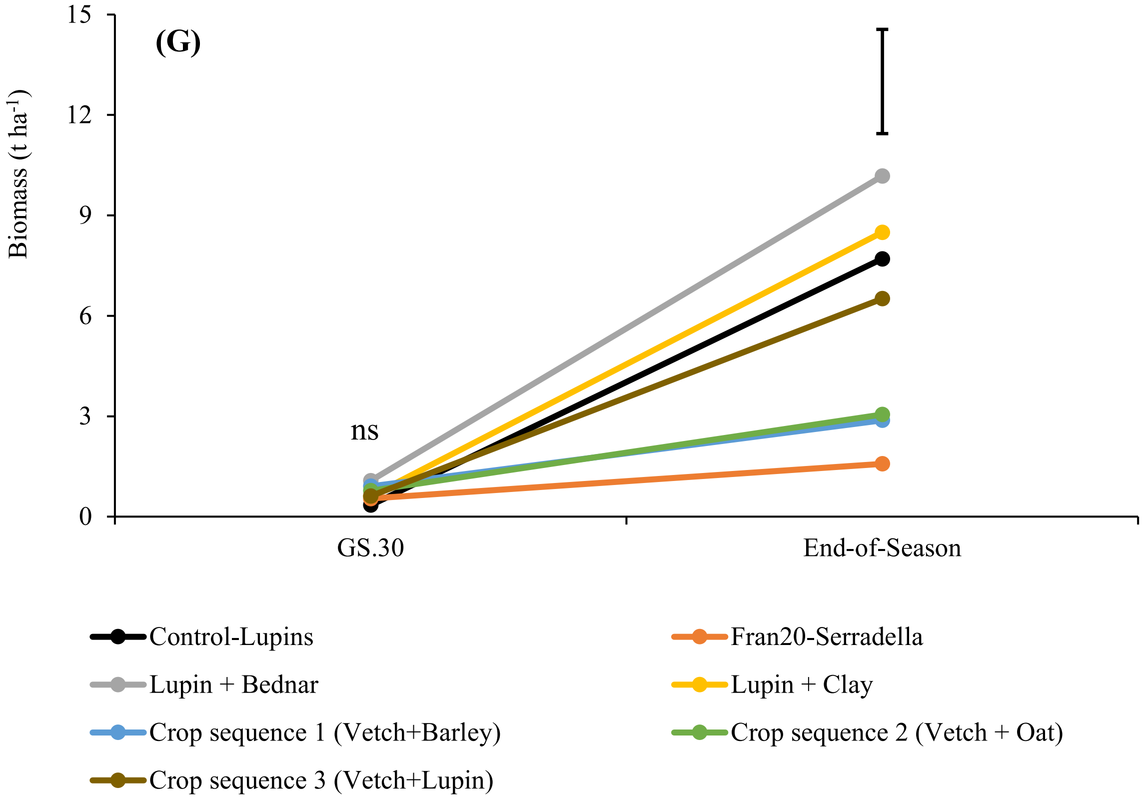
(B)
NDVI at GS.14 (6 weeks)
0.3
0.2
0.1
0.0
Control-Lupins
Fran20-Serradella
Lupin + Bednar
Lupin + Clay
Crop sequence 1 (Vetch+Barley)
Crop sequence 2 (Vetch + Oat)
Crop sequence 3 (Vetch+Lupin)


(C)
NDVI at GS.30 (11 weeks)
0.4
0.3
0.2
0.1
0.0
Control-Lupins
Fran20-Serradella
Lupin + Bednar
Lupin + Clay
Crop sequence 1 (Vetch+Barley)
Crop sequence 2 (Vetch + Oat)
Crop sequence 3 (Vetch+Lupin)


(D)
NDVI
0.6
0.5
0.4
0.3
0.2
0.1
0.0
GS.14 (6 weeks)
GS.30 (11 weeks)
Control-Lupins
Fran20-Serradella
Lupin + Bednar
Lupin + Clay
Crop sequence 1 (Vetch+Barley)
Crop sequence 2 (Vetch + Oat)
Crop sequence 3 (Vetch+Lupin)


(E)
Biomass at GS.30 (t ha-1)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Control-Lupins
Fran20-Serradella
Lupin + Bednar
Lupin + Clay
Crop sequence 1 (Vetch+Barley)
Crop sequence 2 (Vetch + Oat)
Crop sequence 3 (Vetch+Lupin)


(F)
End of season Biomass (t ha-1)
15
12
9
6
3
0
Control-Lupins
Fran20-Serradella
Lupin + Bednar
Lupin + Clay
Crop sequence 1 (Vetch+Barley)
Crop sequence 2 (Vetch + Oat)
Crop sequence 3 (Vetch+Lupin)



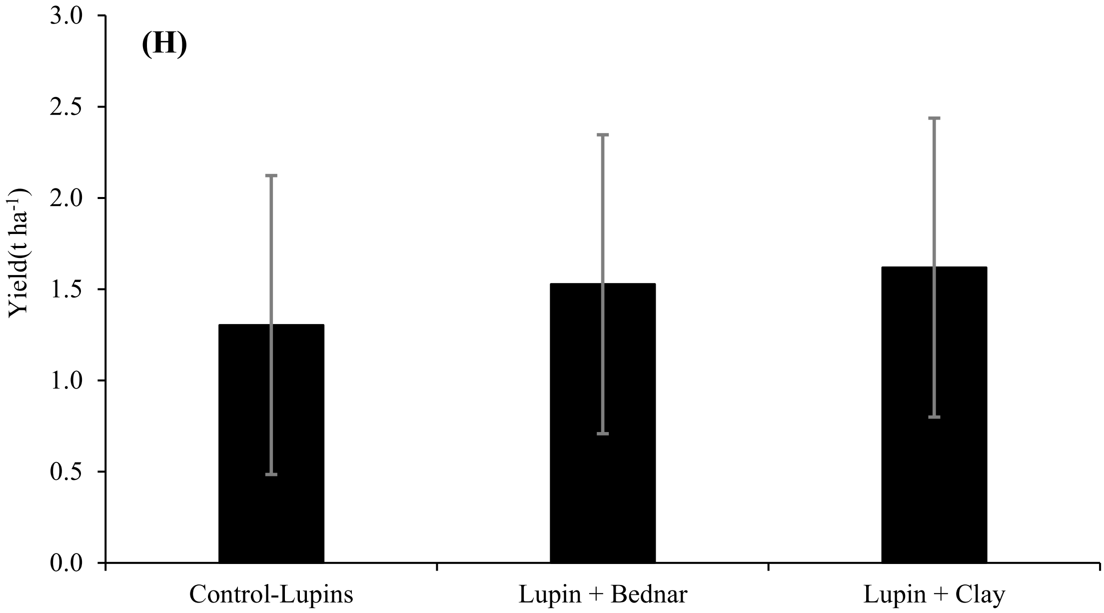


Fig. 5. Effect of different soil and plant-based treatments on **(A)** number of plants m^{-2} **(B)** NDVI at GS.14, **(C)** NDVI at GS.30, **(D)** change in NDVI from GS.14 to GS.30, **(E)** Biomass at GS.30, **(F)** Biomass at the end of season, **(G)** change in biomass from GS.30 to the end of season and **(H)** yield in **2023**. Error bar is the LSD value for comparison of means at 95% level of confidence. Wherever the

treatment effect is significant at α = 95%, LSD has been given as a single error bar to compare the means, otherwise standard error of the treatment mean (n = 4) has been put as the error bar on each data bar.

3.3. 2024 Crop Results

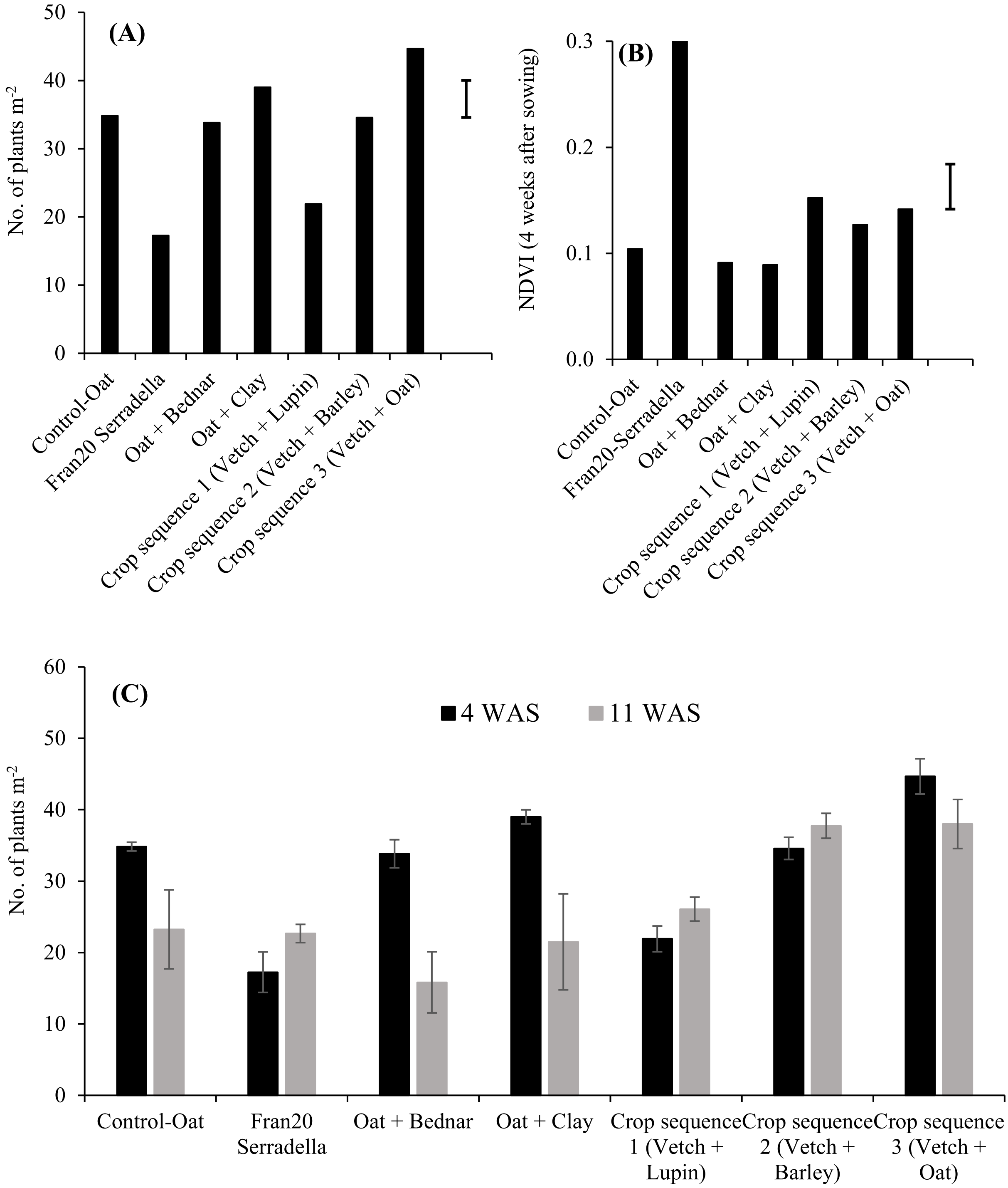
No significant differences were found between the Oats sown on non-treated (control) and treated soil in terms of the plants count and NDVI recorded at 4 weeks after seeding (Fig. 6A-B). However, an issue of Oats plants drying/burning/wilting was found in the 7th week after seeding (4th July 2024) by the Facey Group. The issue was mainly found in the Oats seeded plots (Fig. 6C-D). To quantify the magnitude of the destruction, plants were recounted by the Facey Group in the 11th week after seeding.

The plants count data recorded at 4 weeks after seeding (before damage occurred) and 11 weeks after seeding (after the damage occurred) indicate a significant damage in all the three main treatments, i.e. Control-Oats, Oats sown on Bednar treated plots and Oats sown on clay ameliorated plots. Figures 6C and 6D compare the number of plants m^{-2} at 4 weeks after seeding (before damage) and 11 weeks after seeding (after damage). Figure 6C shows the average number of plants m^{-2} in each treatment before and after the issue. Figure 6D shows the average number of plants m^{-2} in each plot before and after the issue. The plants count recorded at 4 weeks after seeding (WAS) shows that there was an average of 36 ± 3 Oat plants m^{-2} in the control, bednar treated soil and the clay ameliorated soil (Fig 6C). Control Oat plants were reduced by 33%, i.e., 11 less plants m^{-2} than before damage (Figure 6C). This reduction occurred in all the replicates except replicate 1 (Plots 3), i.e. reduction/damage occurred in Replicate 2 (Plot 10), Replicate 3 (Plot 18) and Replicate 4 (Plot 23) with Rep 4 being the most affected one (Figure 6D). Oat plants sown in Bednar treated plots were reduced by 53%, i.e., 18 less plants m^{-2} than before damage (Figure 6C). Reduction occurred in all the replicates i.e. in Replicate 1 (Plot 1), Replicate 2 (Plot 14), Replicate 3 (Plot 19) and Replicate 4 (Plot 27) with Rep 4 being the least affected (Figure 6D). Oat plants sown in clay-ameliorated soil were reduced by 45%, i.e., 17 less plants m^{-2} than before damage (Figure 6C). Reduction occurred in all the replicates i.e. in Replicate 1 (Plot 4), Replicate 2 (Plot 11), Replicate 3 (Plot 20) and Replicate 4 (Plot 25) with little to no reduction in Rep 1-2 and severe effect in Rep 3-4 (Figure 6D). Interestingly, Serradella plants have significantly increased at 11 weeks after seeding relative to 4 weeks after seeding (Figure 6C) perhaps because of more regeneration over time.

Since the crop was affected by the burning issue, particularly the Oat plots. Therefore, the biomass and yield data were analysed using two ways, data as t/ha and as g/m^2. To convert the data into g/m^2, data received per m^2 was divided by the number of plants per m^2 recorded later in the season, i.e., well after the burning issue. In term of t/ha, Control-Oats, Oat+Clay and Sequence-3 (Vetch + Oat) produced greater biomass than the rest of the treatments (Fig. 7A). Thus among the various crop mixes and Serradella treatments, Vetch+Oat produced greater biomass (Fig. 7A).

In term of g/plant, Oat+Clay treatment (64 g/plant) produced the greatest biomass among all the treatments while serradella produced the least (15.6 g/plant), as shown in Fig. 7B. All the three mixes were statistically alike (Fig. 7B). These results are in alignment with the 2024 pre-seeding soil data as the soil sampled from Clay-ameliorated soil was found with significantly greater Nitrate N, greater K colwell, greater organic C (%), greater conductivity, greater exchangeable Ca, much greater exchangeable Mg, greater exchangeable Na, greater Boron Hot $CaCl_2$, and greater DTPA Mn (Fig. 9 & 10).

Yield and protein content data was not significantly affected by the crop (Fig. 8A-C). This could be because of minimal rainfall received in the crucial last 10 weeks of the growing season (18th to 27th week of growing season). The total rainfall received in the last 10 weeks of the growing season was merely 32 mm (Fig. 3) and the crop received no rainfall in 17th, 18th, 21st, 23rd week of the growing season (Fig. 3). This might have limited the ability of the crop to benefit from the soil treatment. This might be the reason that the difference in the biomass (Fig. 7B) did not reflect in the yield as the dryness might have restricted the ability of the crop to transport the dry matter to their grain part.


(A)
No. of plants m-2
0
10
20
30
40
50
Control-Oat
Fran20 Serradella
Oat + Bednar
Oat + Clay
Crop sequence 1 (Vetch + Lupin)
Crop sequence 2 (Vetch + Barley)
Crop sequence 3 (Vetch + Oat)
(B)
NDVI (4 weeks after sowing)
0.0
0.1
0.2
0.3
Control-Oat
Fran20-Serradella
Oat + Bednar
Oat + Clay
Crop sequence 1 (Vetch + Lupin)
Crop sequence 2 (Vetch + Barley)
Crop sequence 3 (Vetch + Oat)
(C)
4 WAS
11 WAS
No. of plants m-2
0
10
20
30
40
50
60
Control-Oat
Fran20 Serradella
Oat + Bednar
Oat + Clay
Crop sequence 1 (Vetch + Lupin)
Crop sequence 2 (Vetch + Barley)
Crop sequence 3 (Vetch + Oat)

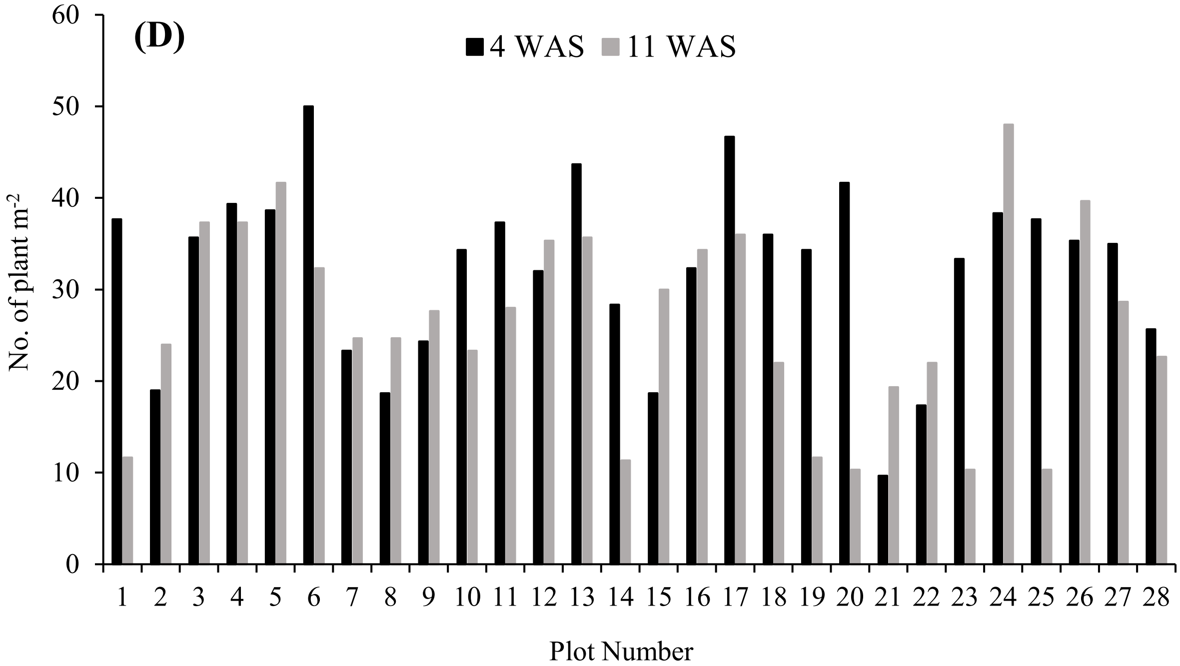


Fig. 6. (A) No. of plants m^{-2} (4 weeks after seeding, i.e., 11th June 2024). **(B)** Green Seeker NDVI at 4 weeks after seeding. **(C)** Comparison between the average number of plants m^{-2} in each treatment recorded at 4 weeks after seeding (before plants burning issue) and 11 weeks after seeding (after plants burning issue). **(D)** Comparison between the average number of plants m^{-2} in each plot recorded at 4 weeks after seeding (before plants burning issue) and 11 weeks after seeding (after plants burning issue). The single error bar in plot A and B represents the LSD for the comparison of means at 95% level of significance. Error bar on each data bar in plot C is the standard error of the individual mean of four replications.

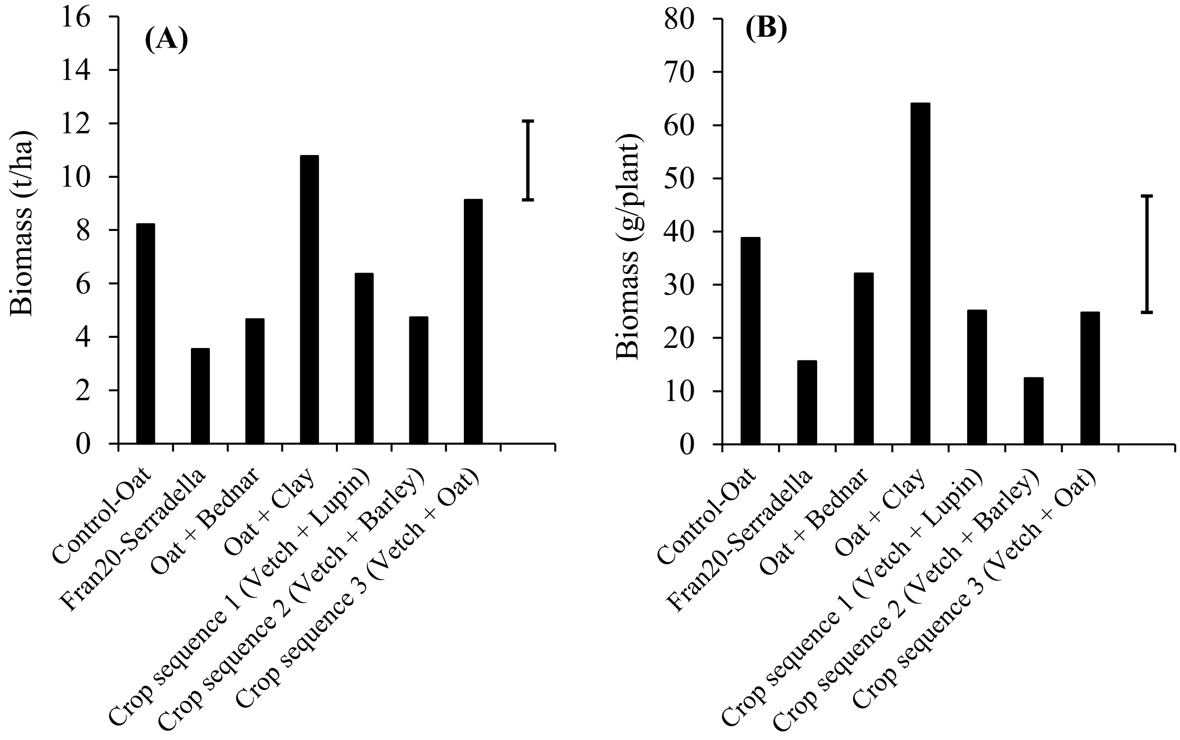
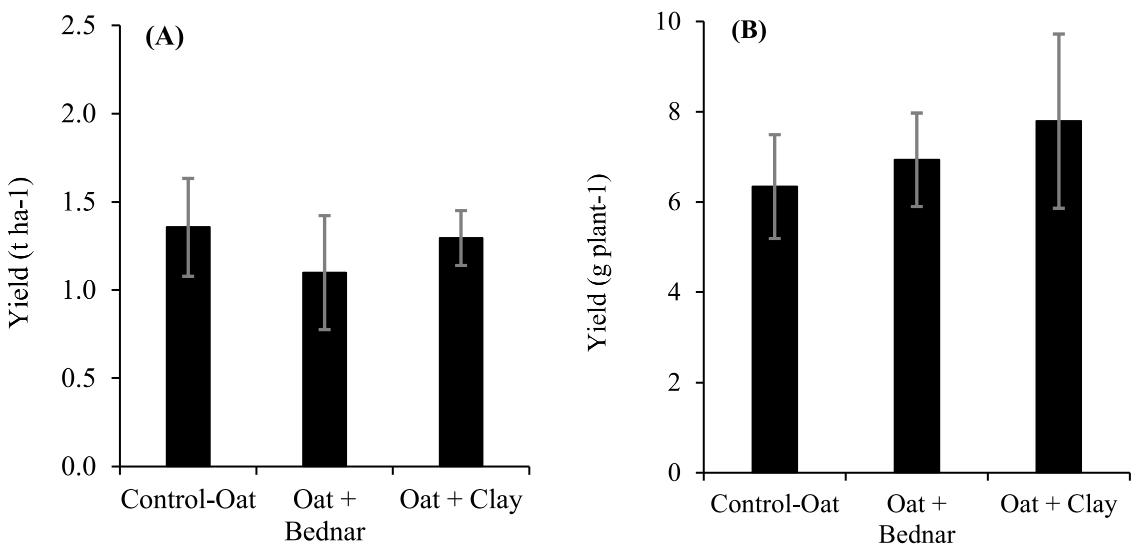


Fig. 7. Biomass of Oat crop recorded in different treatments and sequences at 17 weeks after seeding via manual handcuts. **(A)** Biomass of the various treatments as tons per hectare. **(B)** Biomass of the various treatments converted to grams per plant through dividing the total biomass in grams produced per m^2 by the number of plants recorded per m^2. Error bar represents the LSD for the comparison of means at 95% level of significance.



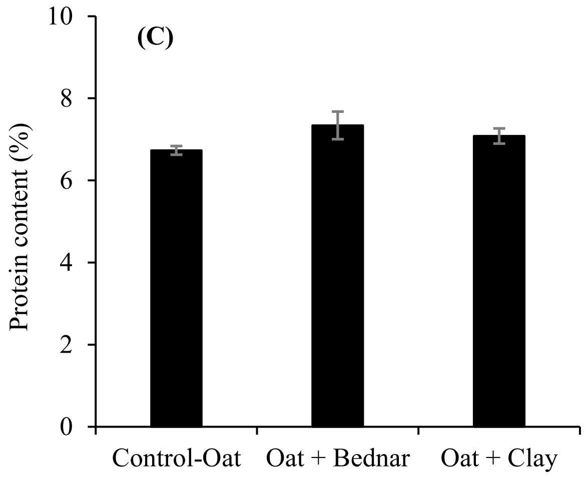


Fig. 8. Harvest data of the crops in various treatments and sequences. **(A)** grain yield of oats received from the various soil treatments as tons per hectare. **(B)** Grain yield of oats received from the various soil treatments converted to grams per plant through dividing the total grains in grams produced per m^2 by the number of plants recorded per m^2. **(C)** protein content in the oat grains (%) received from the various soil treatments. Hence none of these parameters were affected by the treatments significantly, therefore, standard error of the individual mean of four replications has been put as the error bar on each data bar.

3.4. Soil analysis and data (2022-2024)

Soil sampling was done each year to see the trends in soil nutrients and physio-chemical properties over time. In 2022, only the baseline (control) sampling was done. In 2023, soil samples were collected from all the treatments except serradella. In 2024, all the treatments were sampled including serradella at pre-seeding. Samples have been collected from all the treatments in 2025 at pre-seeding which have been sent to the CSBP laboratory and awaiting results. Soil data recorded at pre-seeding in 2024 along with the available data for other years was analysed and graphed to compare the changes in the soil properties of 0-10 cm and 10-30 cm profiles over the past 3 years (2022-2024) as shown in Figure 9A-U and Figure 10A-U.

No significant changes were obsereved in the nitrogen except a spike in nitrate nitrogen observed in 2023 in 0-10 cm profile of the soil seeded with lupin + vetch in 2022 which could be partly attributed to Lupins (Fig. 9A-B and 10A-B). No differences between the treatments were found in terms of phosphorus colwell in 2022 and 2023, however, Serradella treatment was found with significantly greater phosphorus relative to control in 2024 followed by sequence 1 which was Barley+Vetch in the prior year (Fig. 9C and 10C). Treatments were found with no differences in terms of potassium colwell and sulfur in 2022-2024 (Fig. 9D-E and 10D-E). Organic carbon was significantly affected only in the top 10 cm profile of soil in 2023 with clay and sequence 1 (Barley+Vetch in the prior year) showing greater carbon than control (Fig. 9F and 10F).

Differnces between the electric conductivity of the treatments were found both in 2023 and 2024, however, only in the top 10 cm profile (Fig. 9G and 10G). Clay ameliorated soil had significantly greater conductivity in the top 10 cm in both years (Fig. 9G). pH of the top 10 cm soil remained unaffected between the treatments in all the observed years, however, pH of the clay-emliorated soil in 10-30 cm profile was found significantly greater than control in 2024 (Fig. 9H-I and 10H-I).

Exchangeable aluminium was found statistically similar between the treatments in both the depths and all years (Fig. 9J and 10J). Exchangeable calcium in top 10 cm of serradella treatment was lower than all the other treatments including control in 2024 (Fig. 9K) and was found greater than control in the 10-30 cm profile of the clay-ameliorated soil in 2024 (Fig. 10K). Exchangeable magnesium of the clay-ameliorated soil was much higher in both the depths in 2023 and 2024 relative to all the other treatments including control (Fig. 9L and 10L). No significant differences was observed between the treatments in terms of the exchangeable potassium in both the depths and years (Fig. 9M and 10M). Clay-ameliorated soil was found with a significantly greater excnangeable sodium in both the depths in 2023 and 2024 relative to all the other treatments including control (Fig. 9N and 10N).

Boron Hot ($CaCl_2$) of the top 10 cm of clay ameliorated soil was significantly greater than all the other treatments including control in 2024, however, remained unaffected in the 10-30 cm profile (Fig. 9O and 10O). PBI remained largley unaffected except serradellay treatment having greater PBI than control in top 10 cm in 2024 (Fig. 9P and 10P).

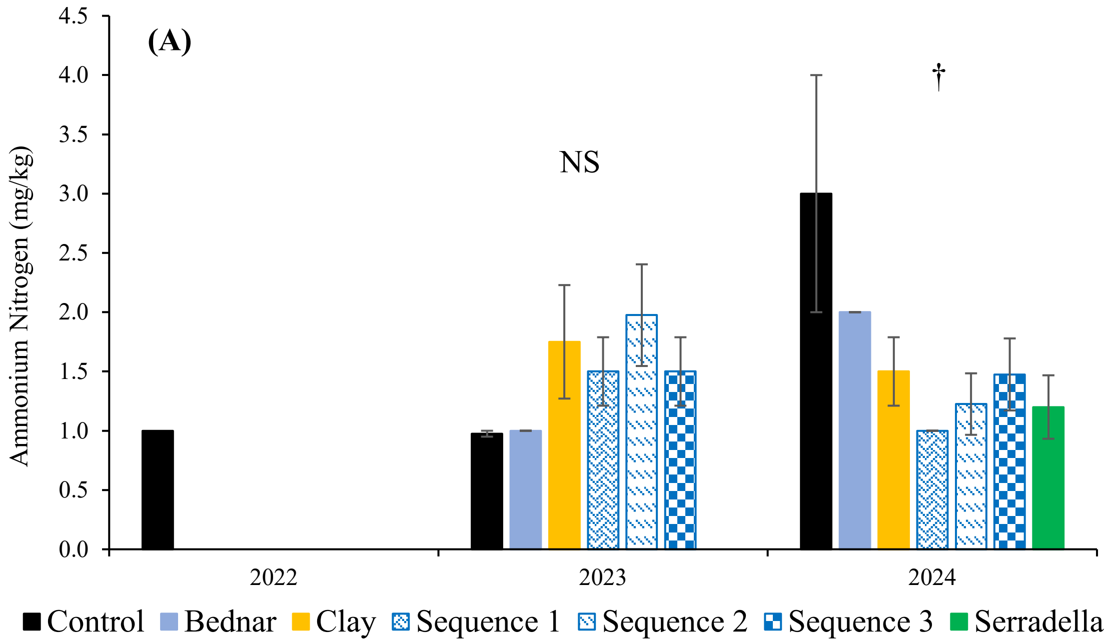
DTPA copper had almost the similar pattern as PBI, however, in addition to serradella having greated copper, sequence 2 and 3 had lesser copper than control in 0-10 cm profile in 2024 (Fig. 9Q and 10Q). DTPA iron was found similar between the treatments in all depths and years (Fig. 9R and 10R). DTPA manganese in the 0-10 cm profile of the clay ameliorated soil was significantly greater than control in 2024 with Serradella having lower than control (Fig. 9S). 10-30 cm profile of the clay-ameliorated soil and sequence 1 were found with significantly greater DTPA manganese in 2023 than control but no differences were observed in the deeper layer in the following year (Fig. 10S). No differences were founnd in the soil in termes of DTPA zinc in both the depths and years (Fig. 9T and 10T).

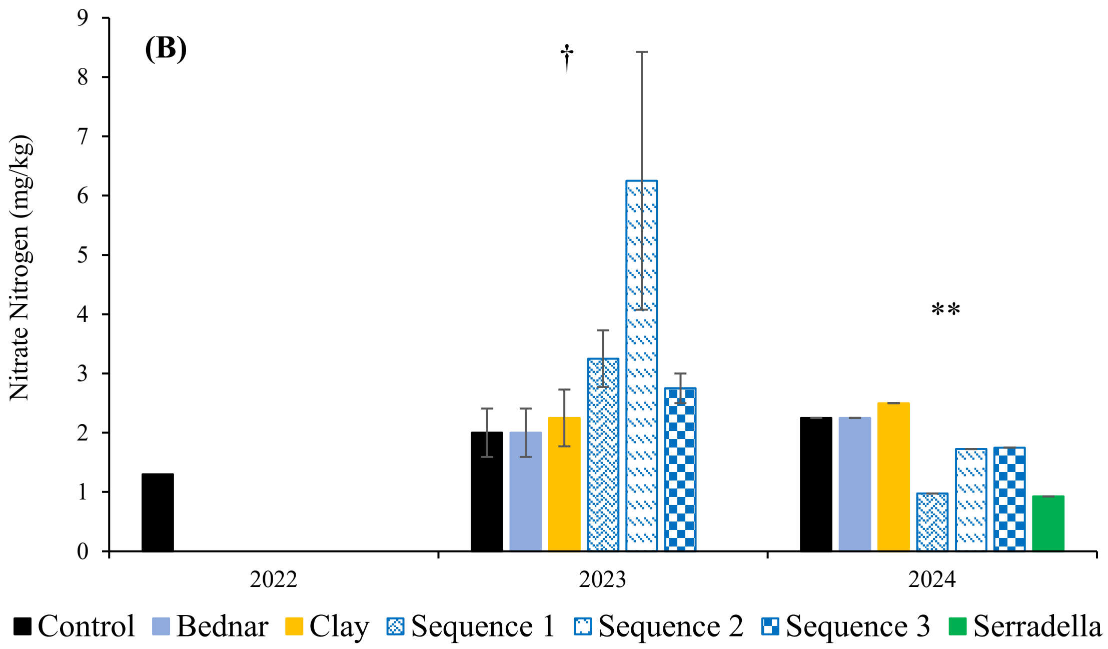
Aluminium ($CaCl_2$) of the 10-30 cm profile was found significantly lower in the clay-ameliorated soil and greater in the serradella treatments relative to control in 2024, however, remained unaffected in the top 10 cm (Fig. 9U and 10U).

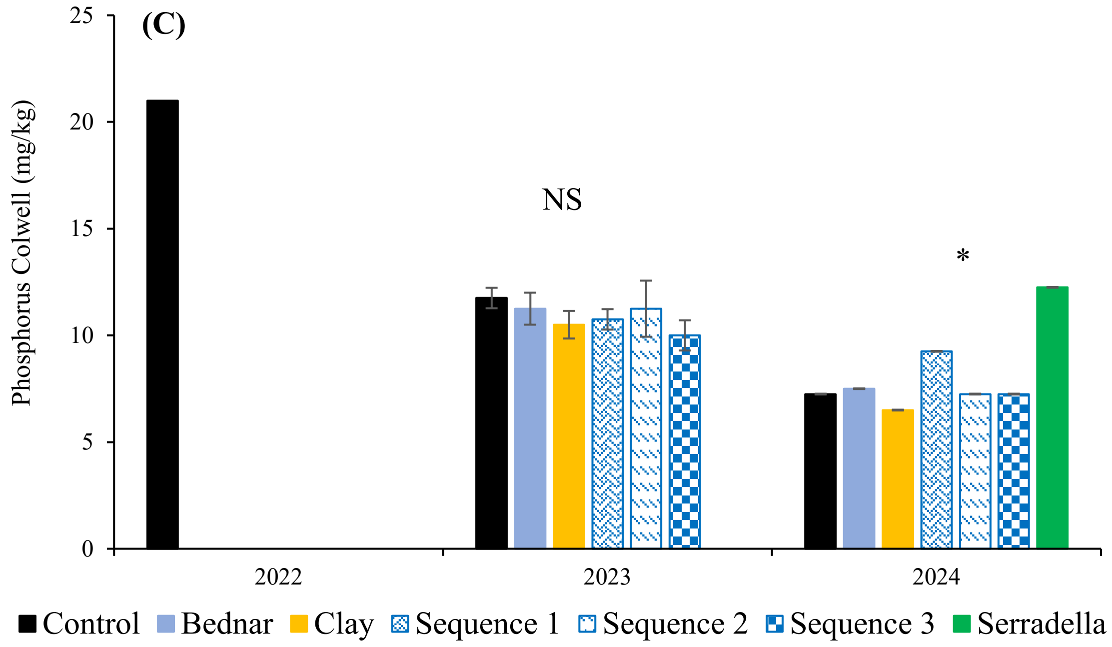
To summarise, among the soil treatments, clay-ameliorated soil was found with more differences relative to control in the top as well as sub-soil. Among the plant-based treatments, serradella was found with more differences relative to other treatments over cnotrol. Relative to 2023, more changes were observed in 2024.

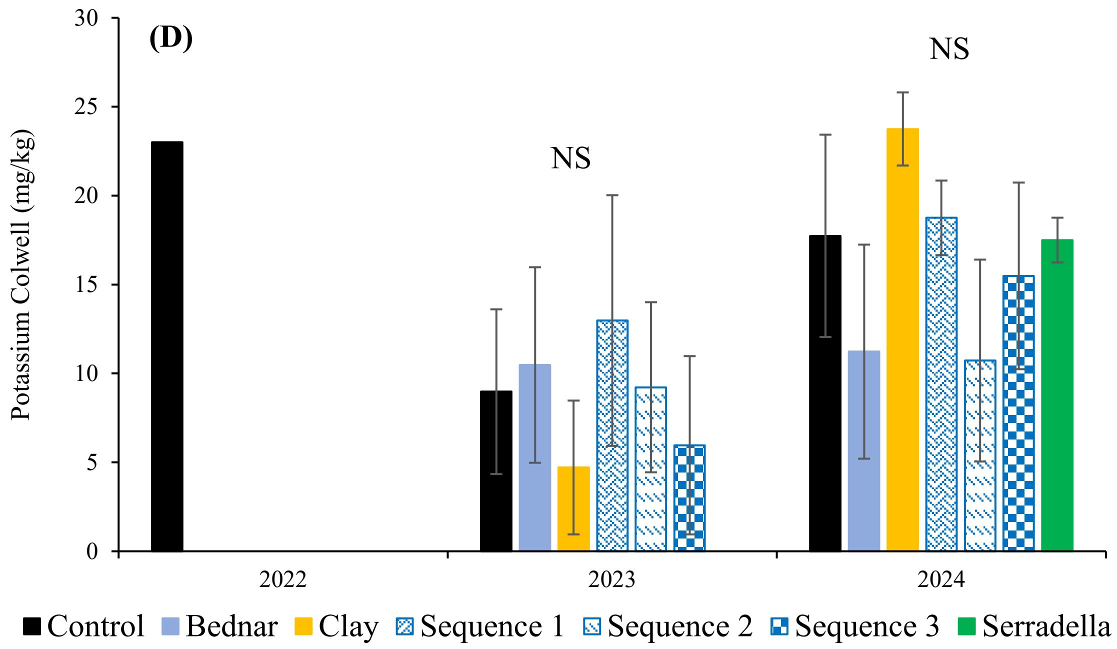
The 2024 crop data along with the soil analysis showed that deep ripping, clay amendments, and crop sequencing influenced soil health and productivity. While clay incorporation improved soil moisture and nutrient retention, late-season drought limited its impact on yield. Serradella and mixed cropping enhanced phosphorus availability, supporting long-term soil fertility. However, increased sodium and magnesium in clay-treated soils highlight the need for careful management. The findings emphasise that soil improvements must be paired with effective water management to maximise benefits, guiding toward more resilient and sustainable farming systems.

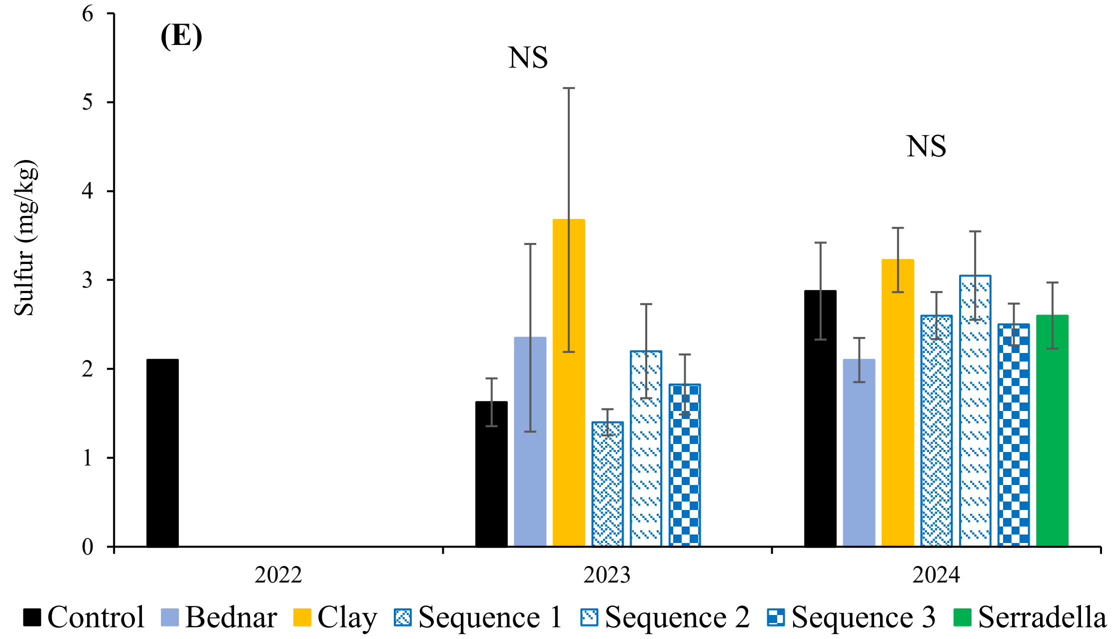
Another batch of samples has been collected from 0-10, 10-30 and 30-45 cm depth at the end of February 2025. Samples have been sent for comprehensive analysis to the CSBP laboratory in Bibra Lake, WA and results are being awaited. In addition, samples for the Predictor B analysis (Soil DNA testing for nematodes by SARDI) have been collected from 0-10 and 10-30 cm depths of the selected treatments (control, bednar, clay and crop sequence 1). Samples have already been delivered to Katherine Linsell at SARDI.


(A)
Ammonium Nitrogen (mg/kg)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
NS
†
2022
2023
2024
Control Bednar Clay Sequence 1 Sequence 2 Sequence 3 Serradella

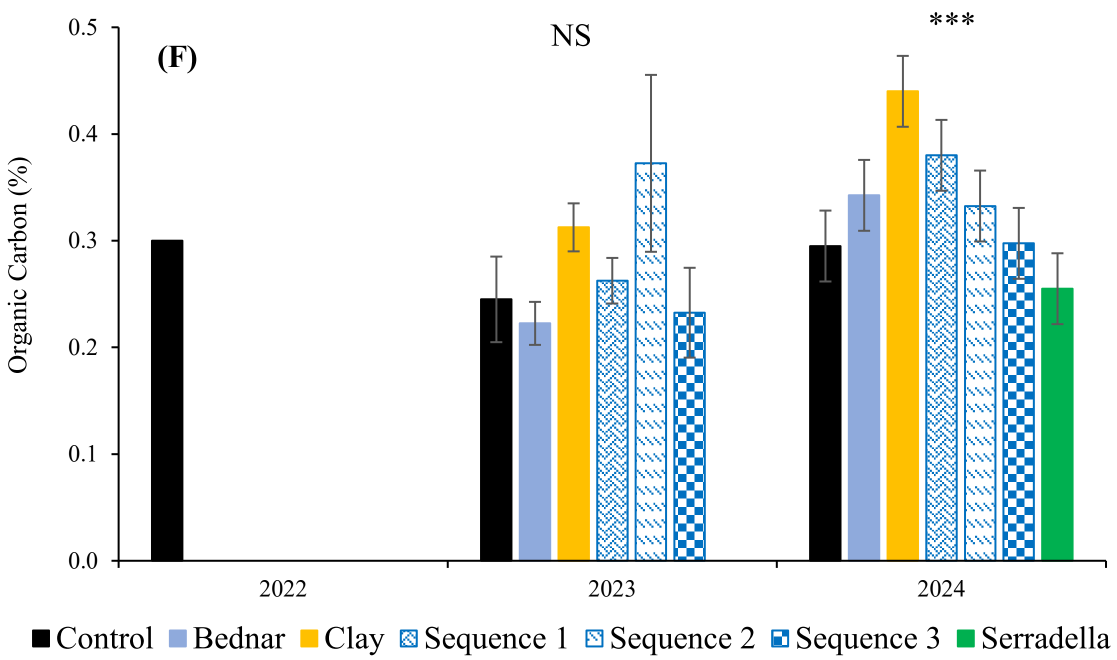

(B)
Nitrate Nitrogen (mg/kg)
9
8
7
6
5
4
3
2
1
0
†
**
2022
2023
2024
Control Bednar Clay Sequence 1 Sequence 2 Sequence 3 Serradella


(C)
Phosphorus Colwell (mg/kg)
25
20
15
10
5
0
NS
*
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

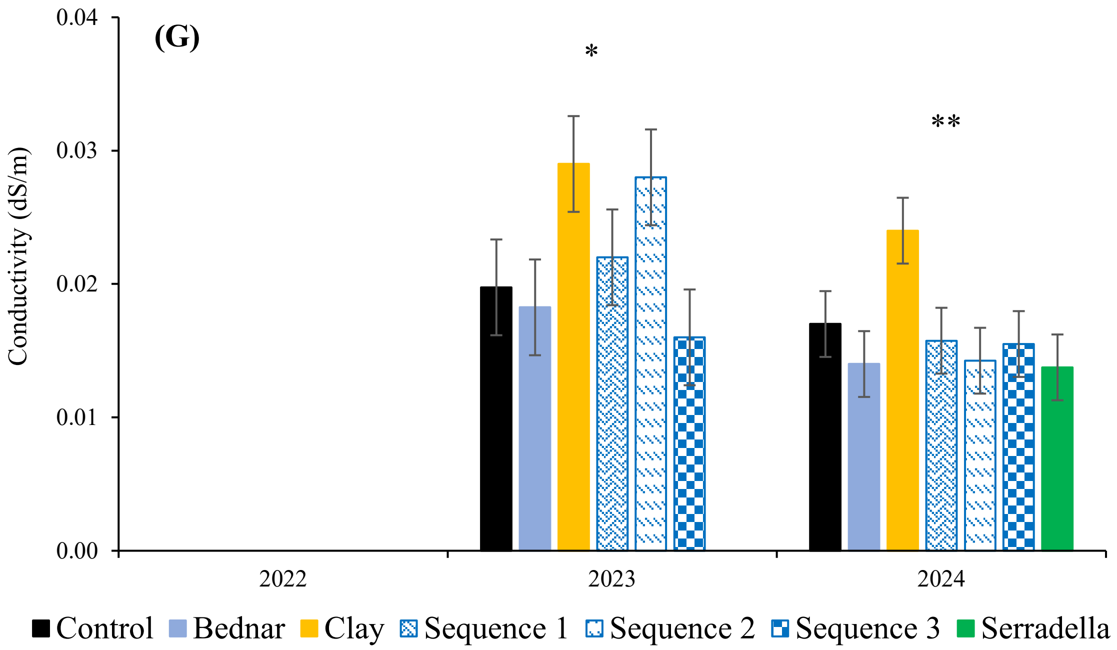

(D)
Potassium Colwell (mg/kg)
30
25
20
15
10
5
0
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



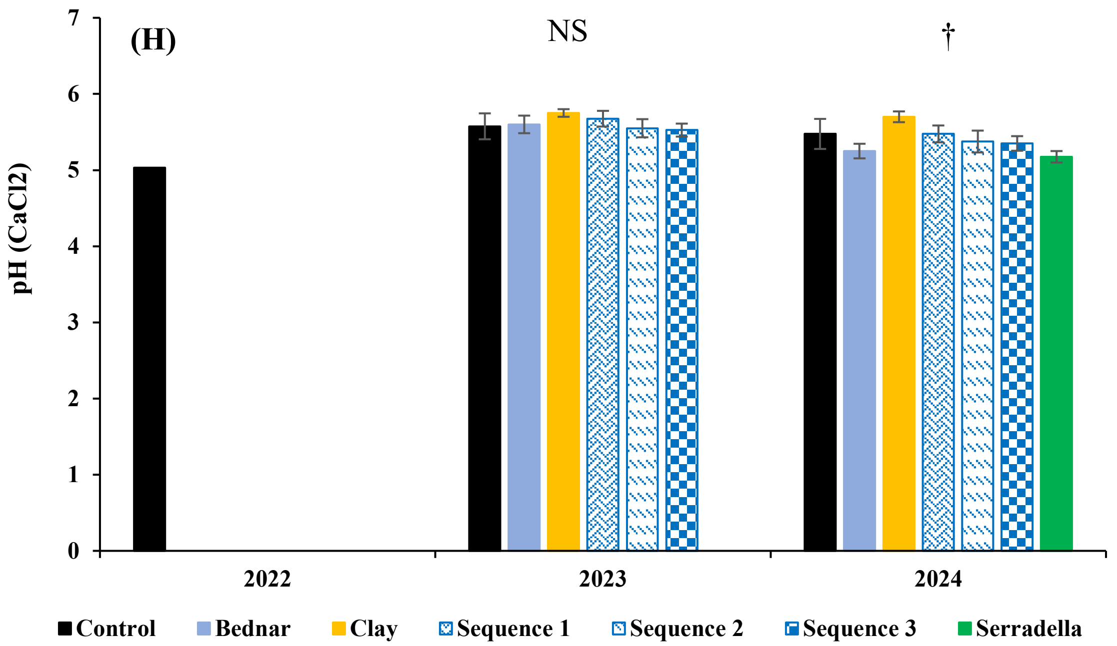
(E)
NS
NS
Sulfur (mg/kg)
0
1
2
3
4
5
6
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



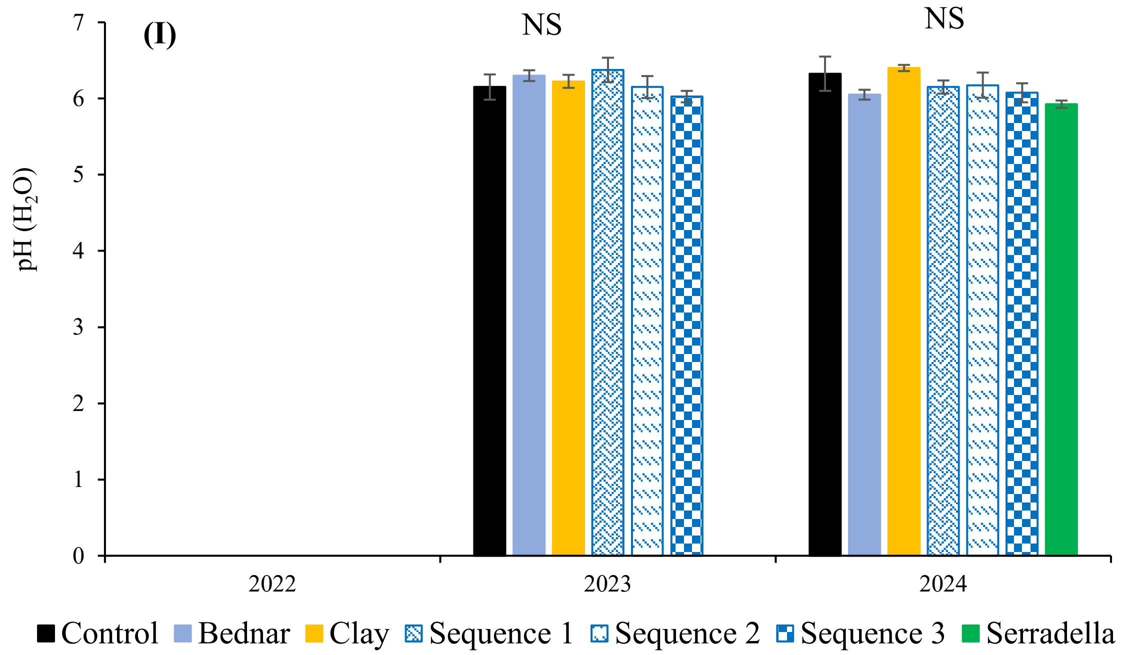
(F)
NS
***
Organic Carbon (%)
0.0
0.1
0.2
0.3
0.4
0.5
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

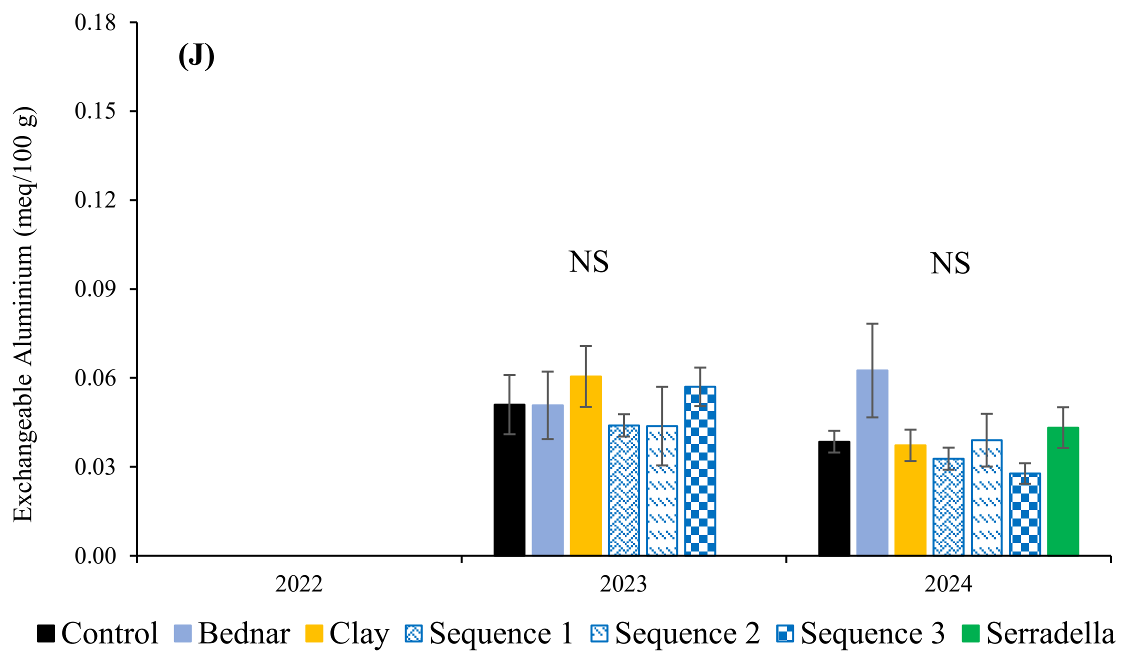


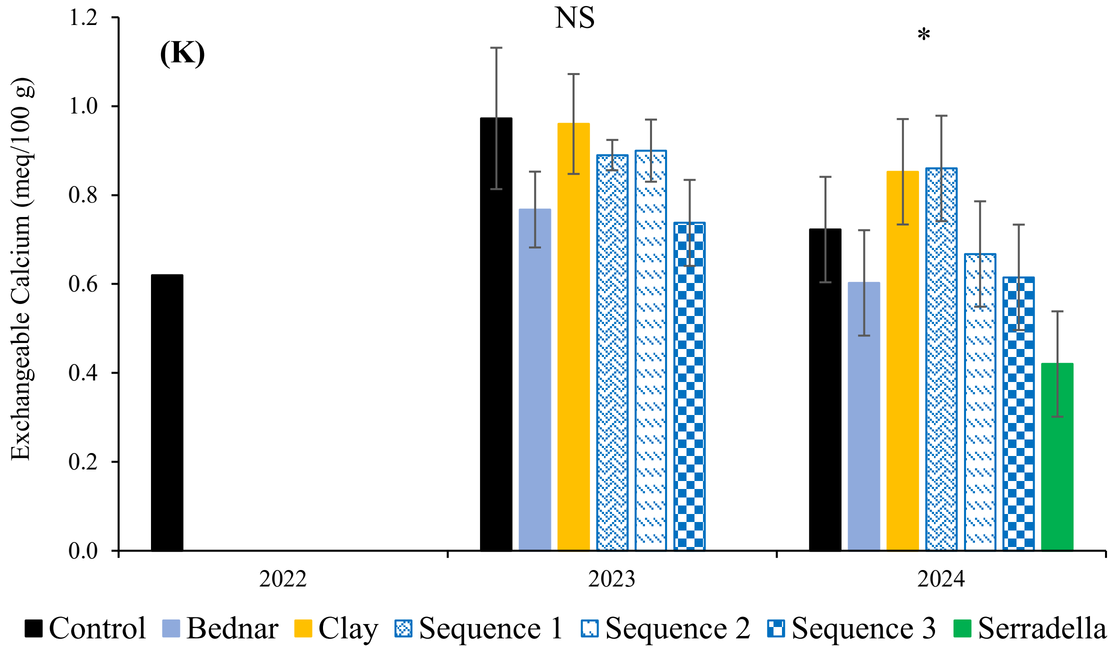
(G)
*
**
Conductivity (dS/m)
0.04
0.03
0.02
0.01
0.00
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

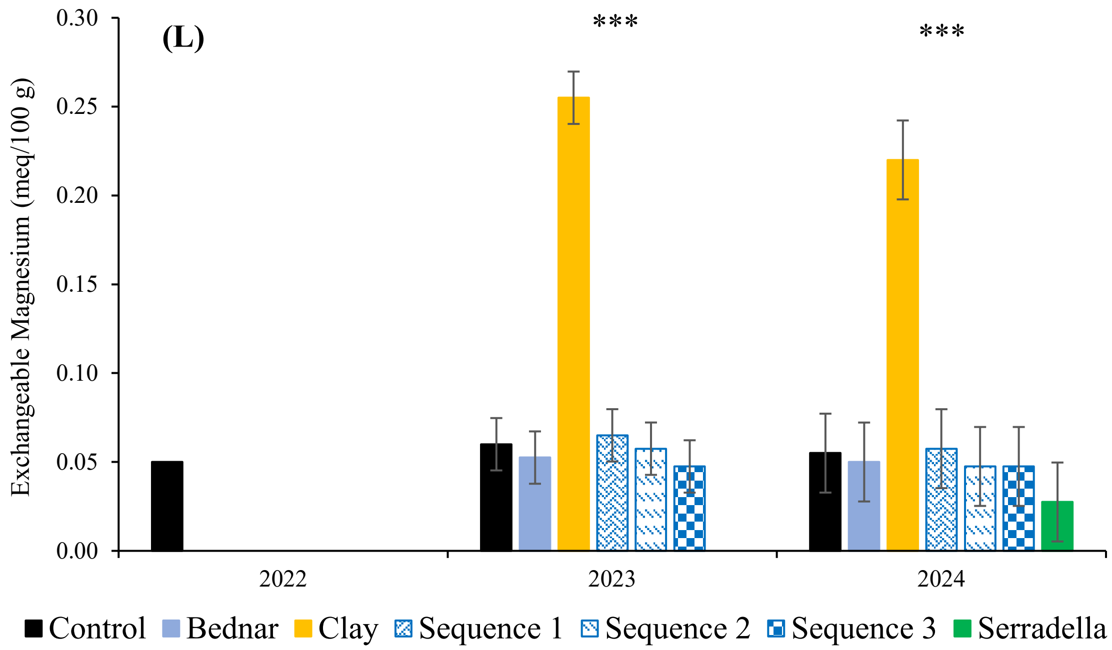


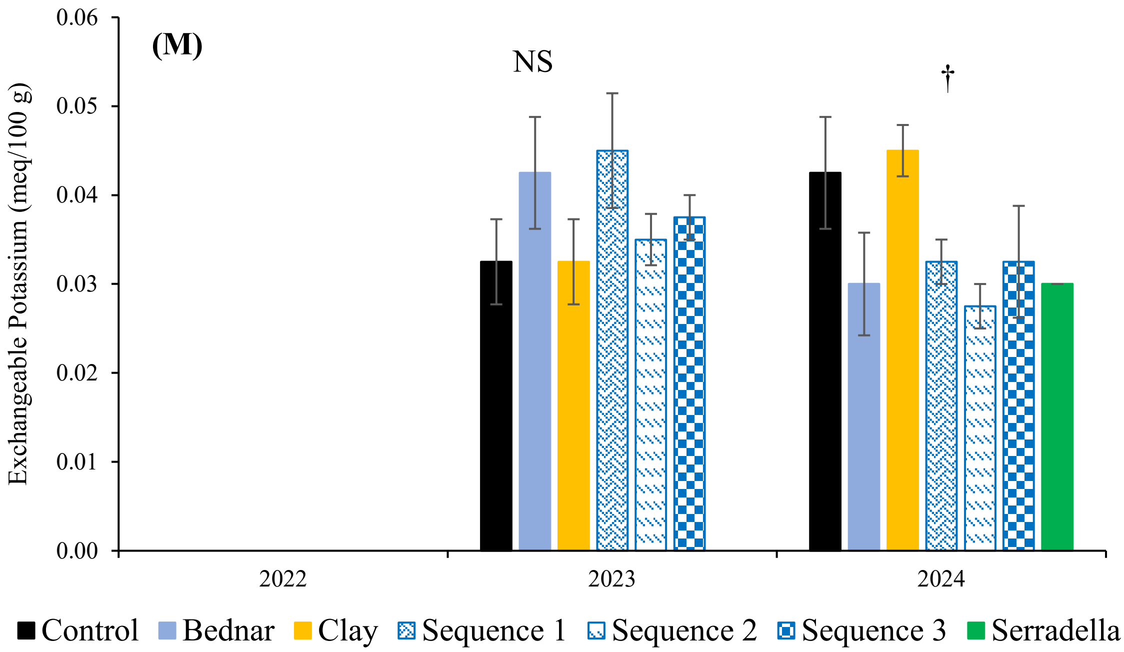
(H)
NS
†
pH (CaCl2)
7
6
5
4
3
2
1
0
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

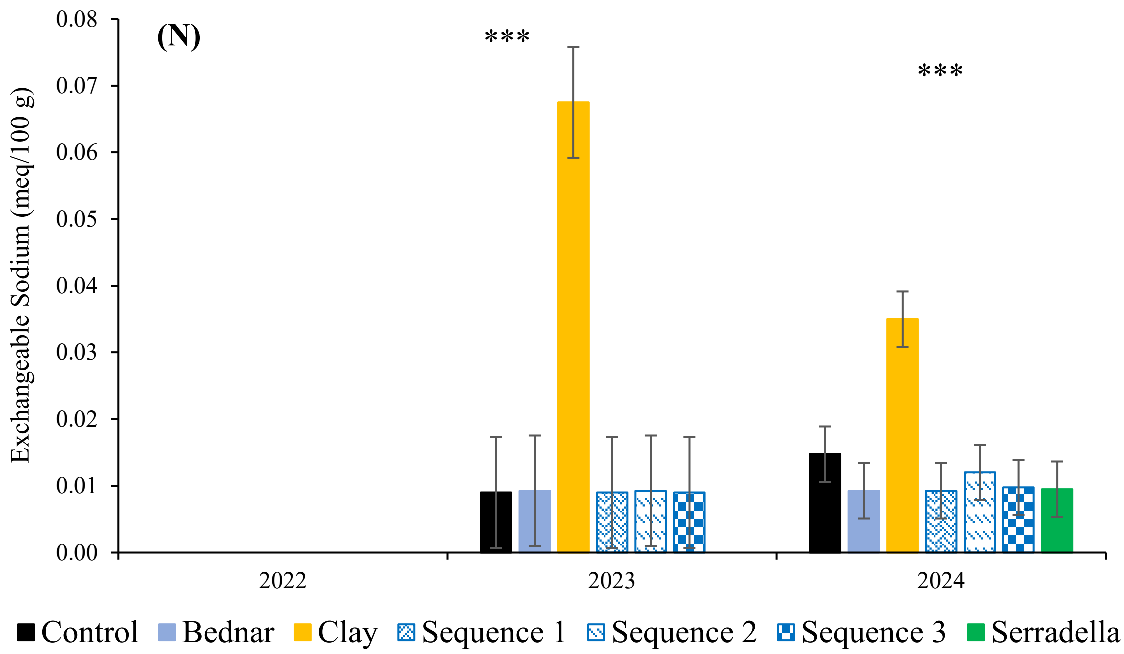

(I)
pH (H_2O)
NS
NS
0
1
2
3
4
5
6
7
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

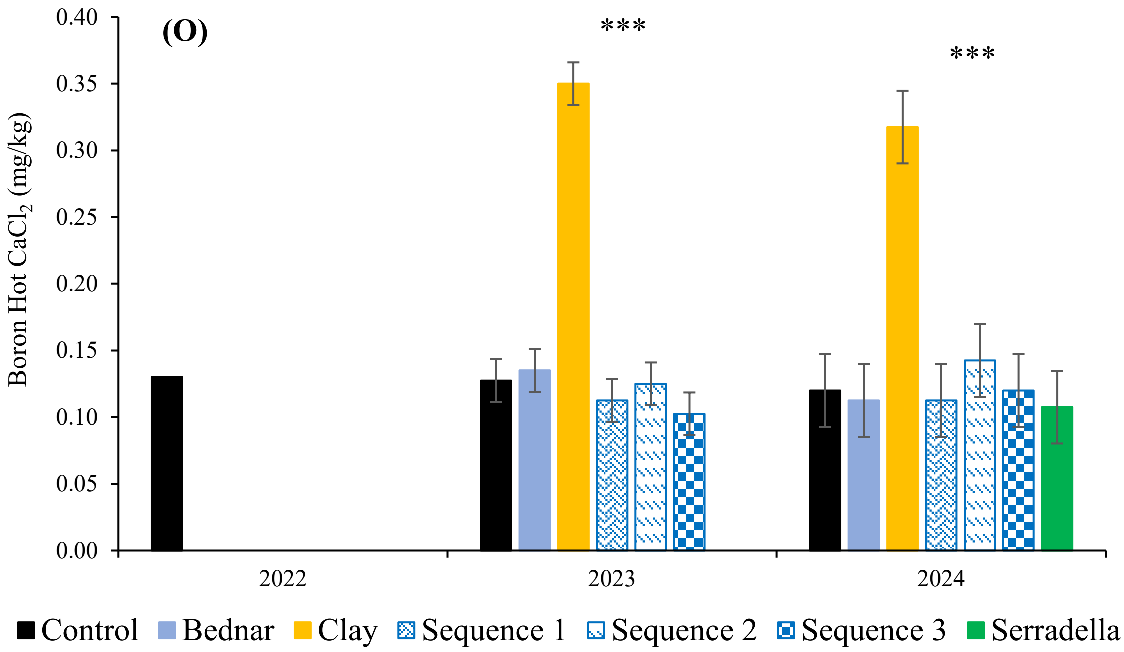

(J)
Exchangeable Aluminium (meq/100 g)
NS
NS
0.00
0.03
0.06
0.09
0.12
0.15
0.18
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


NS
*
(K)
Exchangeable Calcium (meq/100 g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

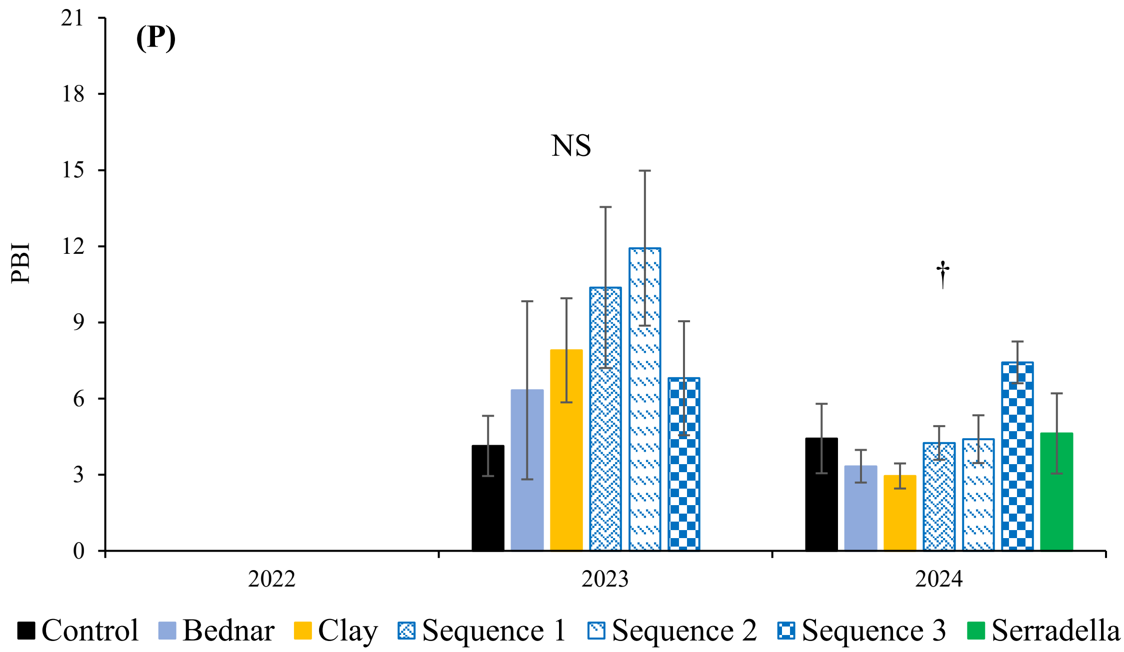

***
***
(L)
Exchangeable Magnesium (meq/100 g)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(M)
NS
†
Exchangeable Potassium (meq/100 g)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

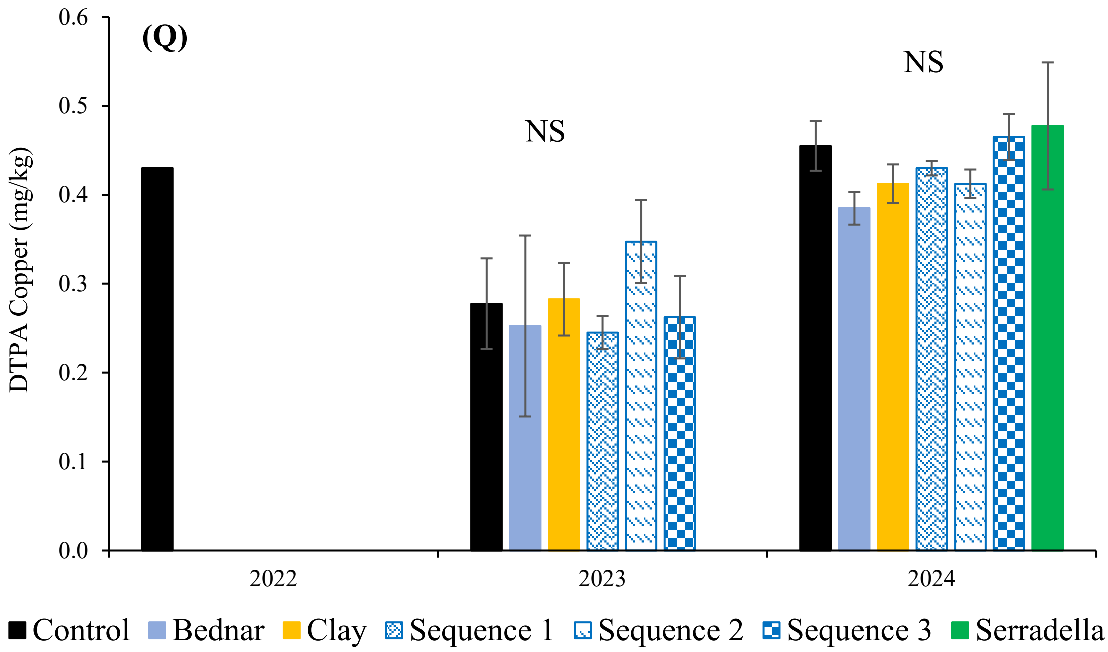

(N)
***
***
Exchangeable Sodium (meq/100 g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

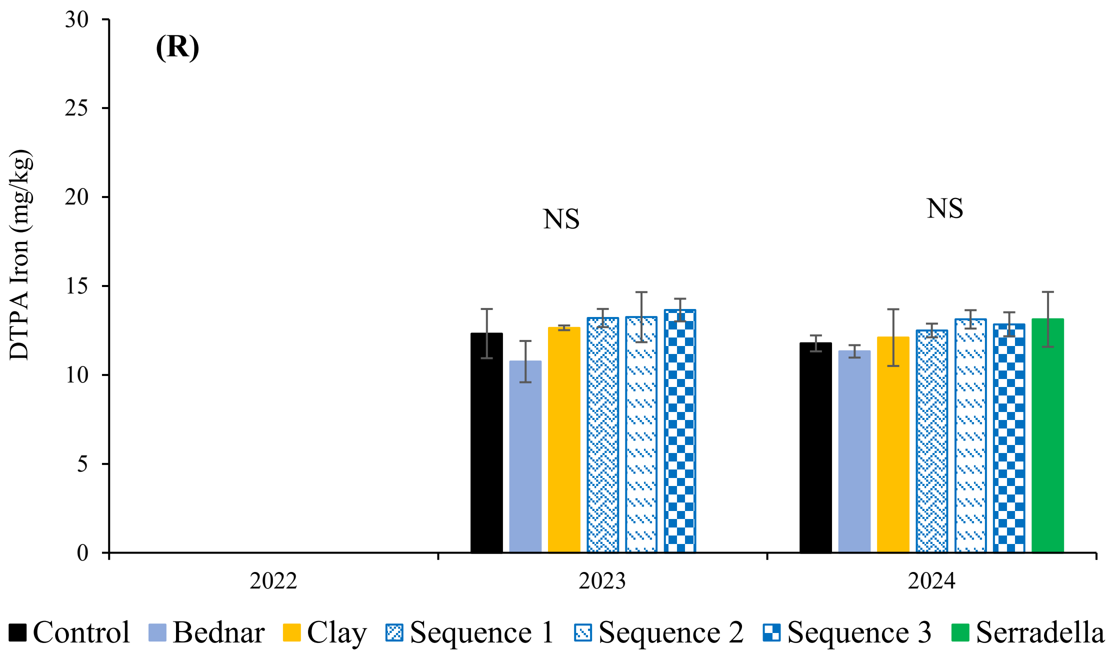


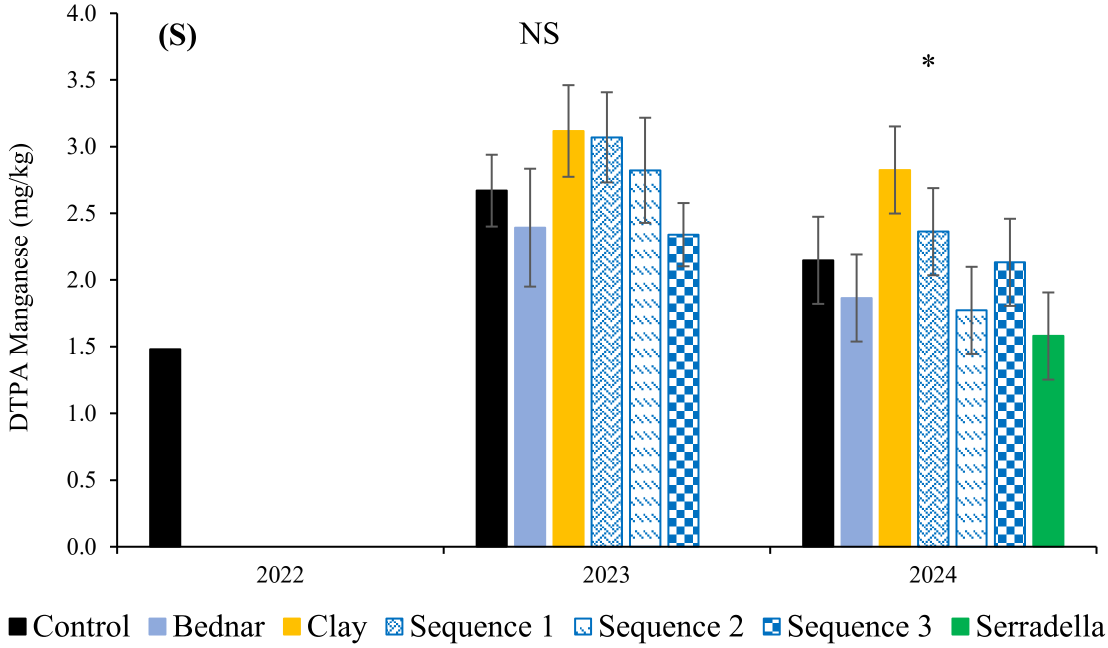
(O)
Boron Hot $CaCl_2$ (mg/kg)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
***
***
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

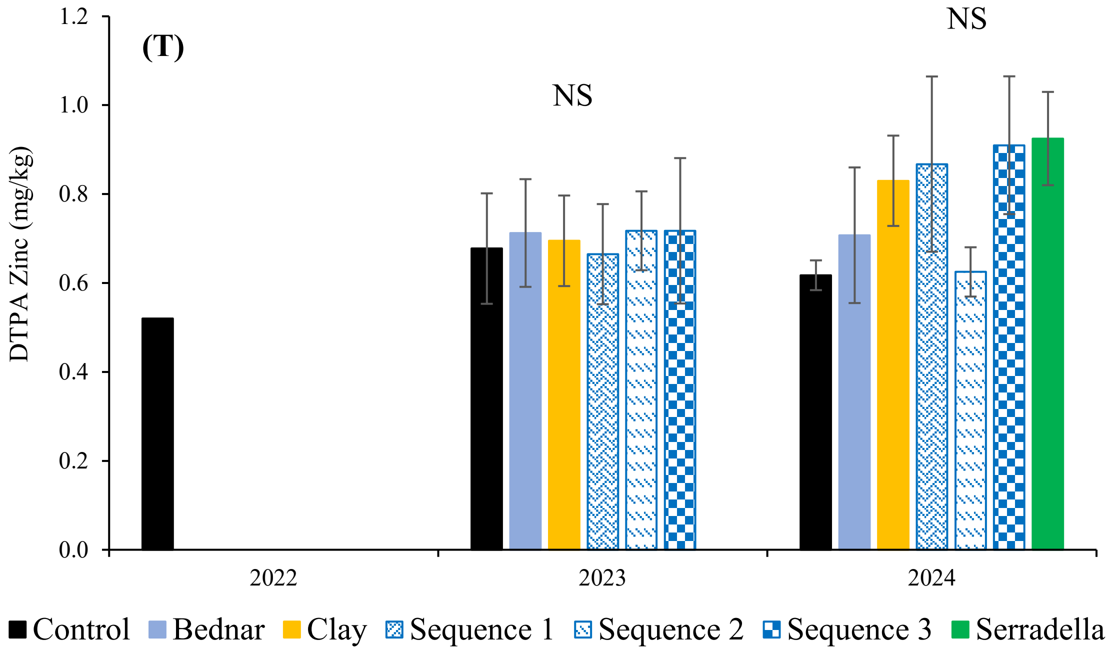


(P)
PBI
21
18
15
12
9
6
3
0
NS
†
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(Q)
DTPA Copper (mg/kg)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(R)
DTPA Iron (mg/kg)
0
5
10
15
20
25
30
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(S)
NS
*
DTPA Manganese (mg/kg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(T)
NS
NS
DTPA Zinc (mg/kg)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

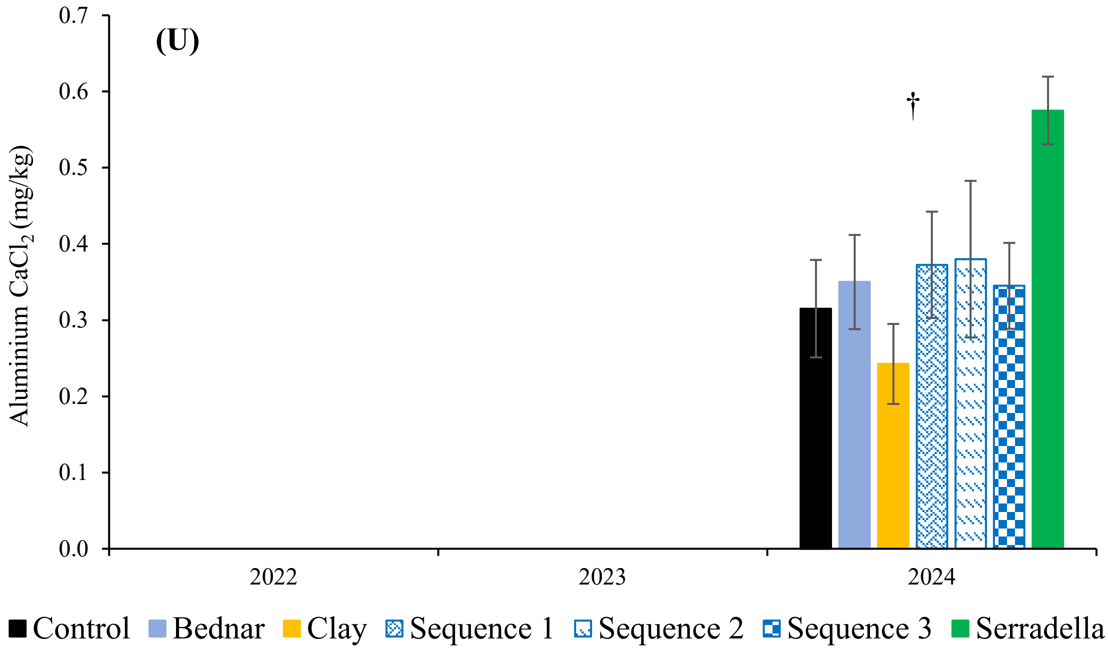
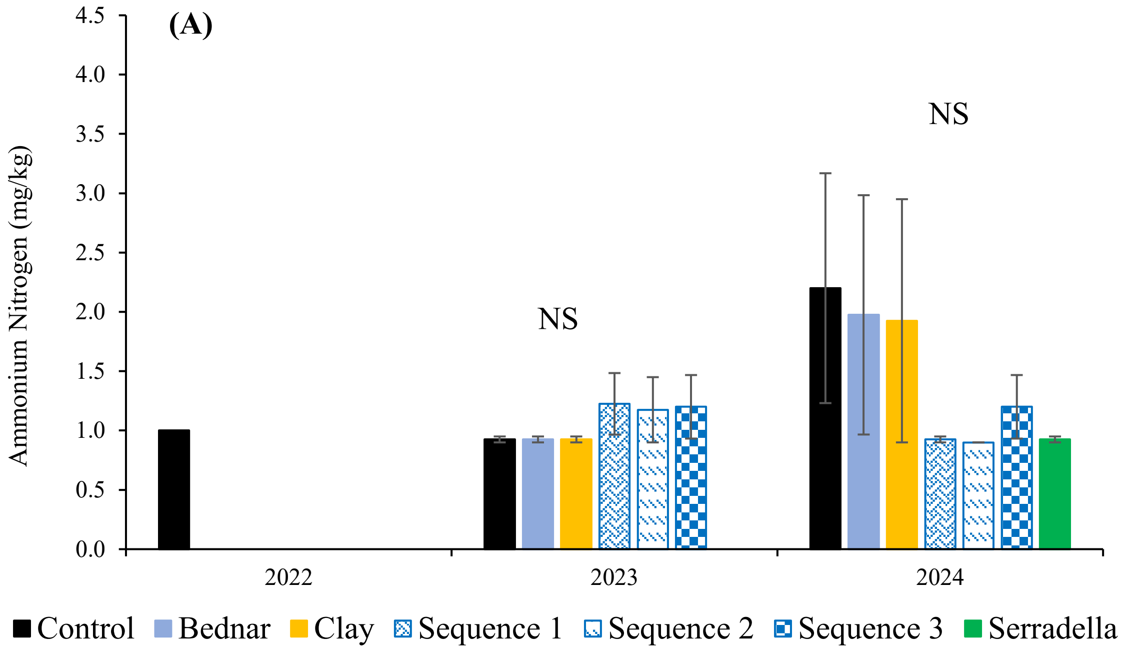


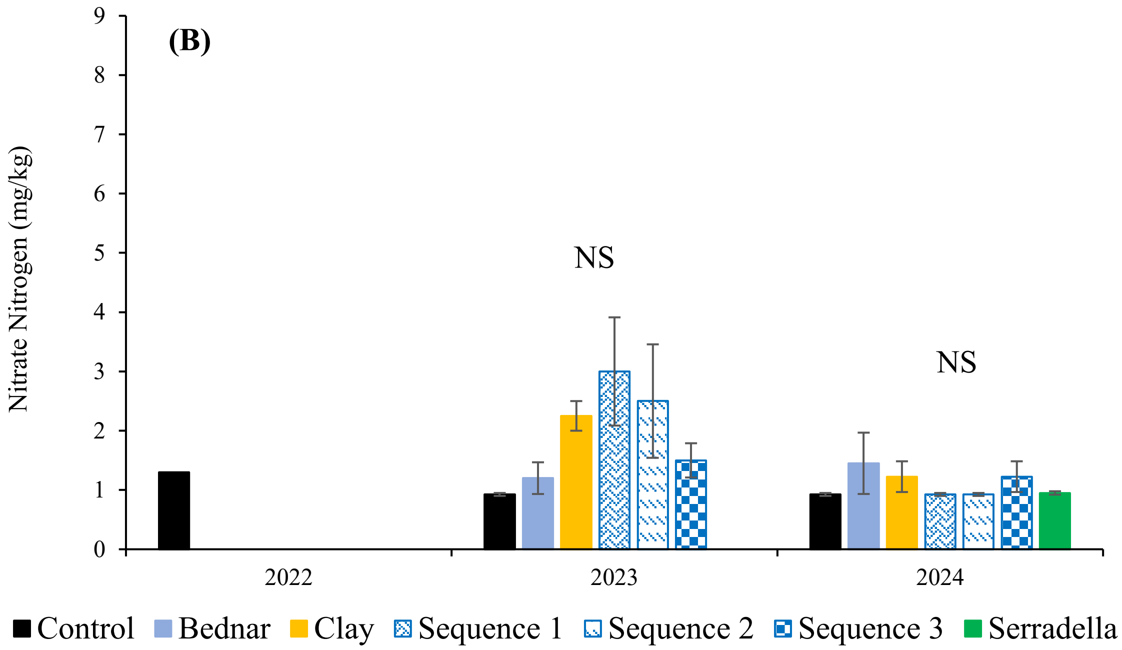
Fig. 9. Soil physio-chemical properties and nutrients status in 0-10 cm profile at Bullaring trial site, recorded at pre-seeding stage in 2024. **(A)** Ammonium Nitrogen (mg/kg). **(B)** Nitrate Nitrogen (mg/kg). **(C)** Phosphorus Colwell (mg/kg). **(D)** Potassium Kolwell (mg/kg). **(E)** Sulfur (mg/kg). **(F)** Organic Carbon (%). **(G)** Conductivity (dS/m). **(H)** pH ($CaCl_2$). **(I)** pH (H_2O). **(J)** Exchangeable Aluminium (meq/100 g). **(K)** Exchangeable Calcium (meq/100 g). **(L)** Exchangeable Magnesium (meq/100 g). **(M)** Exchangeable Potassium (meq/100 g). **(N)** Exchangeable Sodium (meq/100 g). **(O)** Boron hot $CaCl_2$ (mg/kg). **(P)** PBI. **(Q)** DTPA Copper (mg/kg). **(R)** DTPA Iron (mg/kg). **(S)** DTPA Manganese (mg/kg). **(T)** DTPA Zinc (mg/kg). **(U)** Aluminium $CaCl_2$ (mg/kg).

Data of each year have been statistically analysed independent of other years. NS = $P > 0.1$, † = $P < 0.1$, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$. n = 4. Where P is greater than 0.05, standard error of each individual mean has been inserted on each data bar as error bar. Where P is equal to or less than 0.05, a single standard error for the experiment/year has been inserted as error bar on all the data bars for that particular year to compare the means of that year.

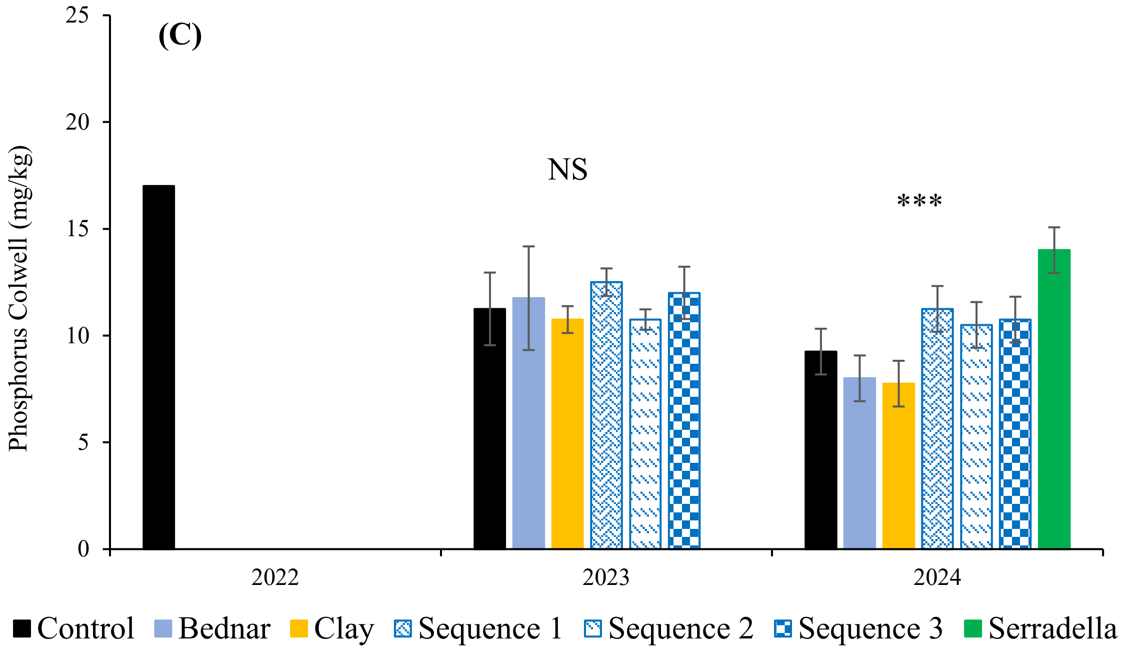
Sequence 1 was Oat+Vetch in 2022, Barley+Vetch in 2023 and Lupin+Vetch in 2024. Sequence 2 was Lupin+Vetch in 2022, Oat+Vetch in 2023 and Barley+Vetch in 2024. Sequence 3 was Barley+Vetch in 2022, Lupin+Vetch in 2023 and Oat+Vetch in 2024.



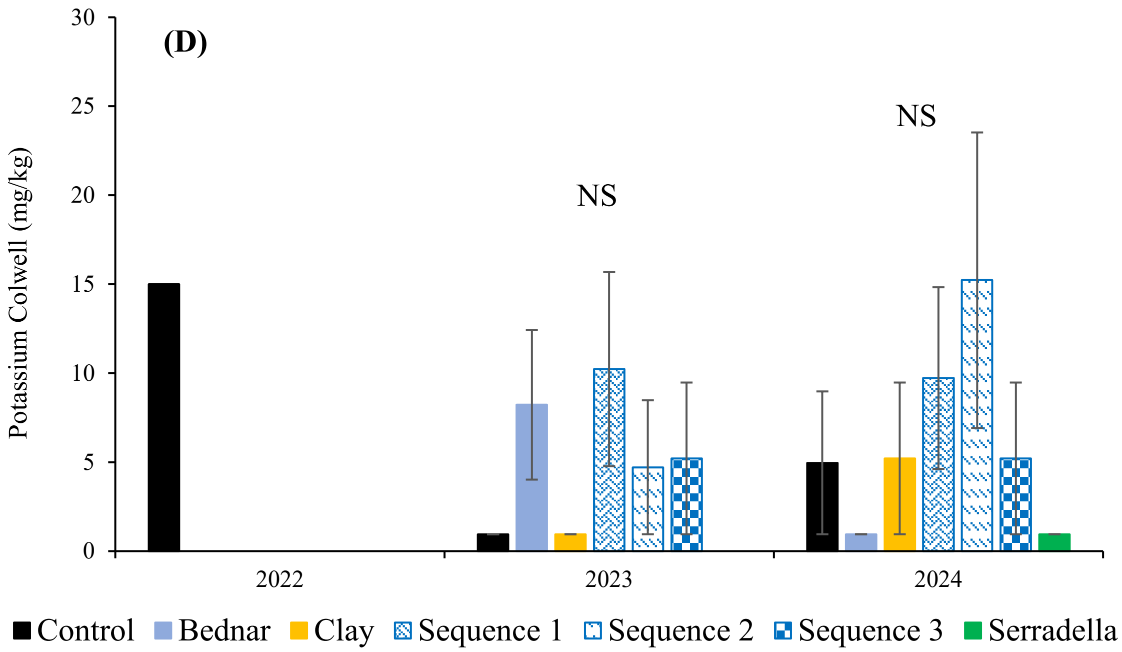
(A)
Ammonium Nitrogen (mg/kg)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



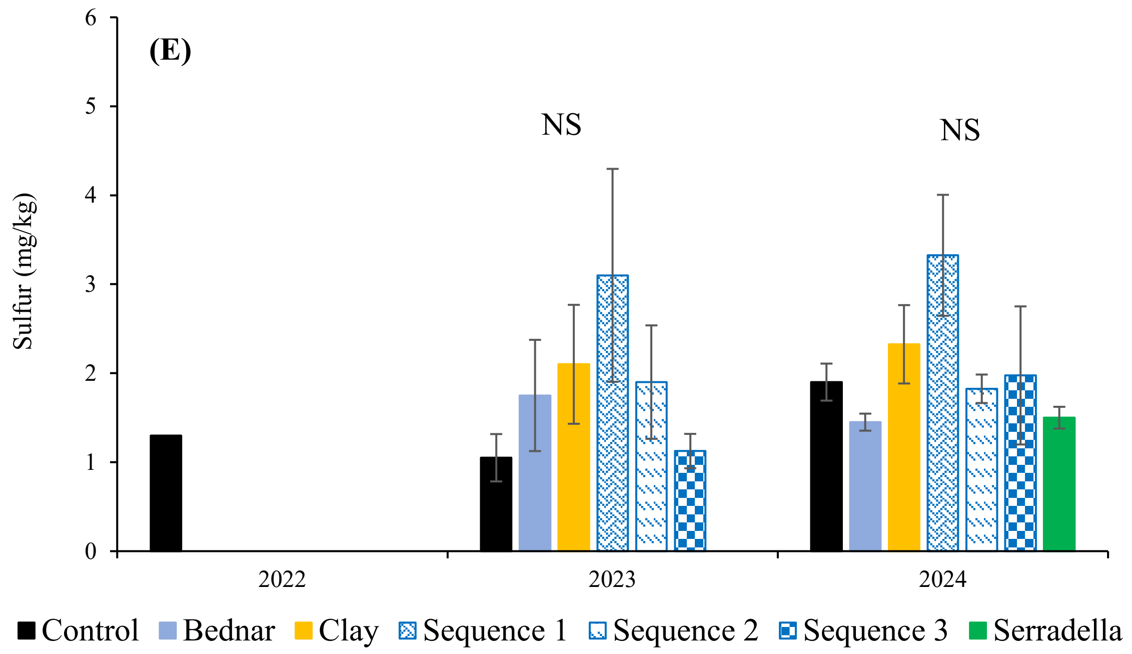
(B)
Nitrate Nitrogen (mg/kg)
9
8
7
6
5
4
3
2
1
0
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

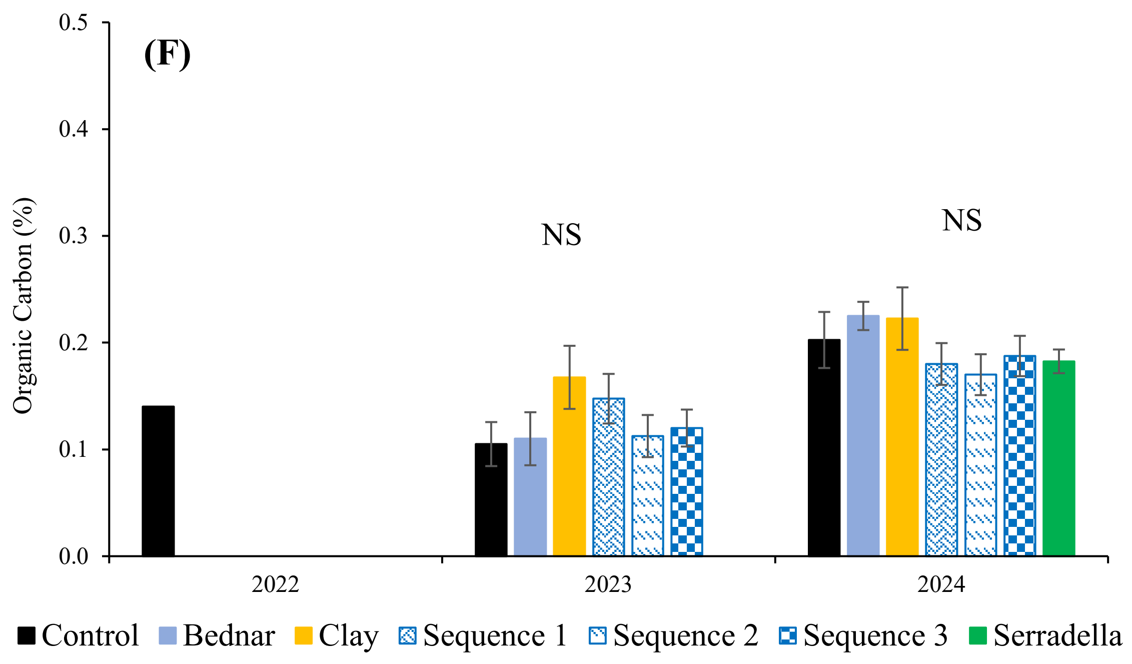


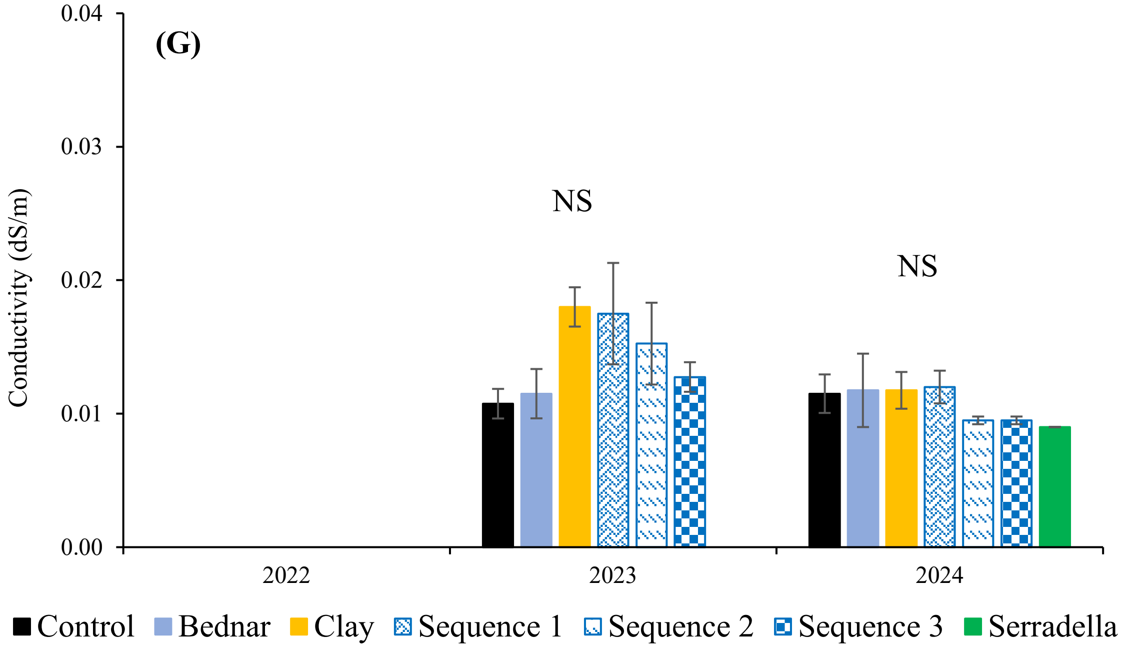
(C)
Phosphorus Colwell (mg/kg)
25
20
15
10
5
0
NS
***
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



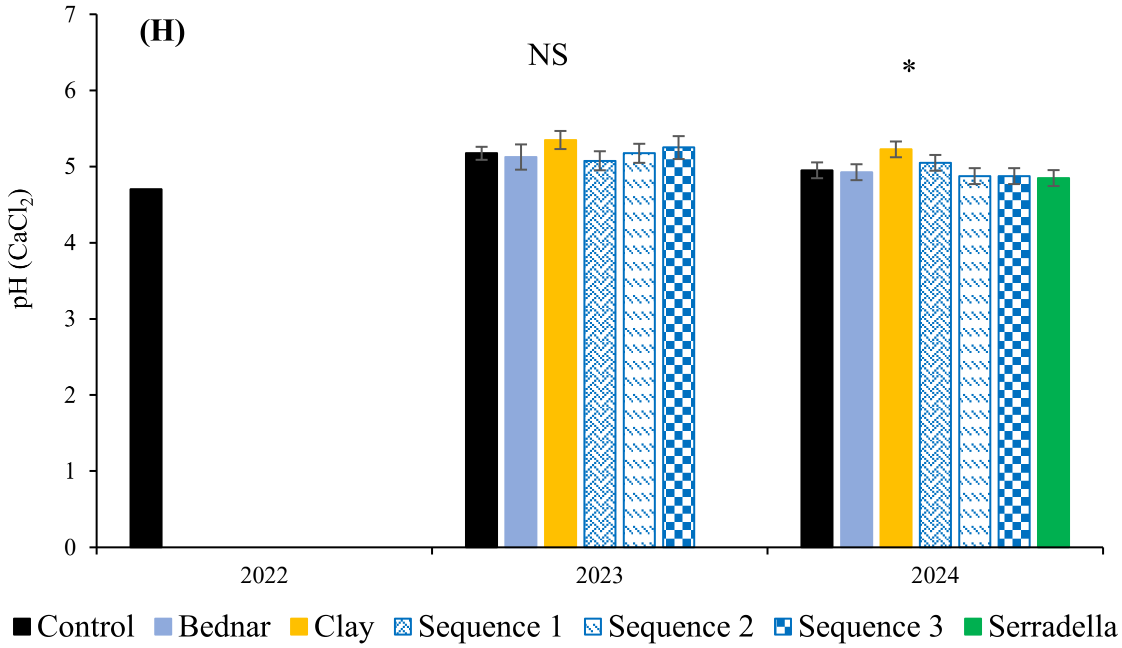
(D)
Potassium Colwell (mg/kg)
30
25
20
15
10
5
0
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(E)
Sulfur (mg/kg)
0
1
2
3
4
5
6
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

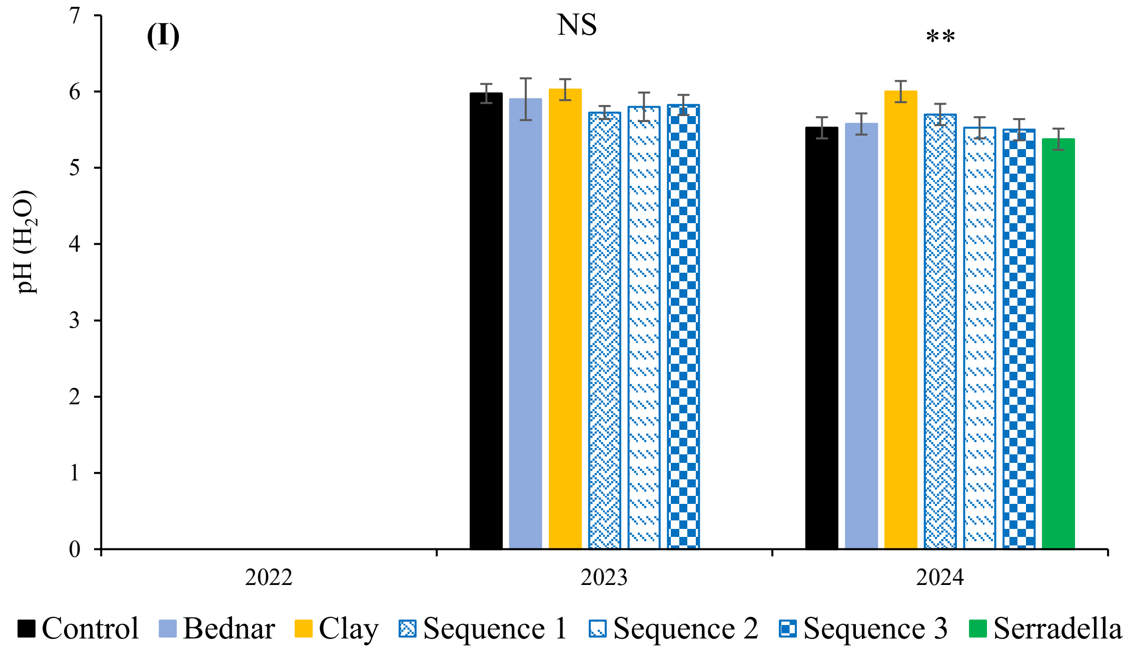

(F)
Organic Carbon (%)
0.0
0.1
0.2
0.3
0.4
0.5
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



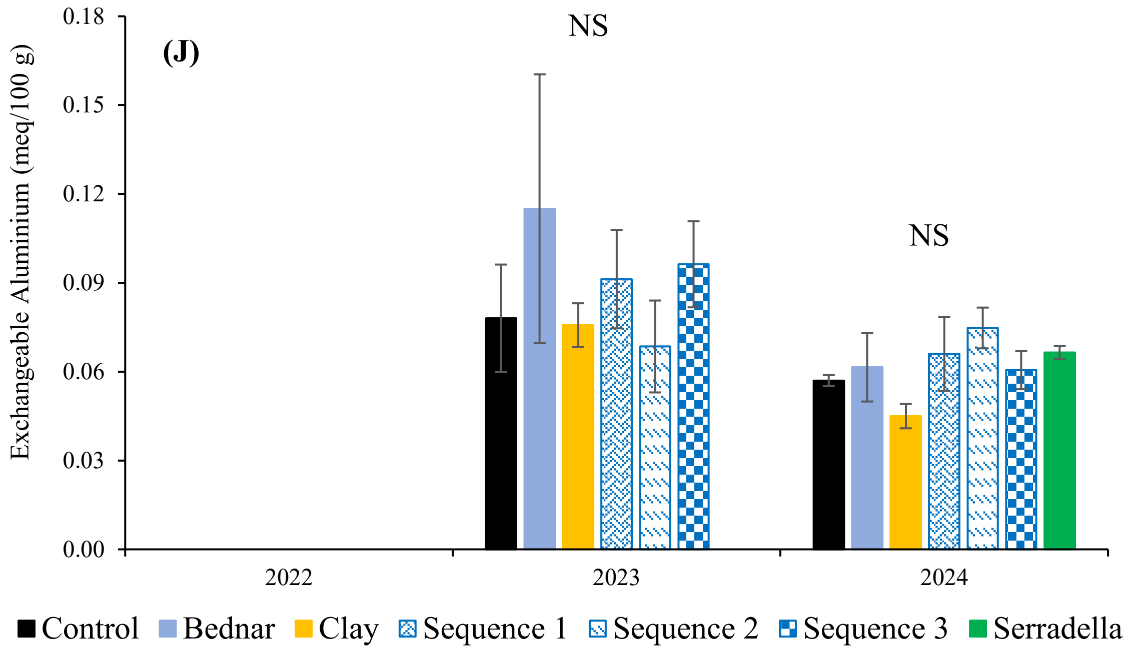
(G)
Conductivity (dS/m)
0.04
0.03
0.02
0.01
0.00
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



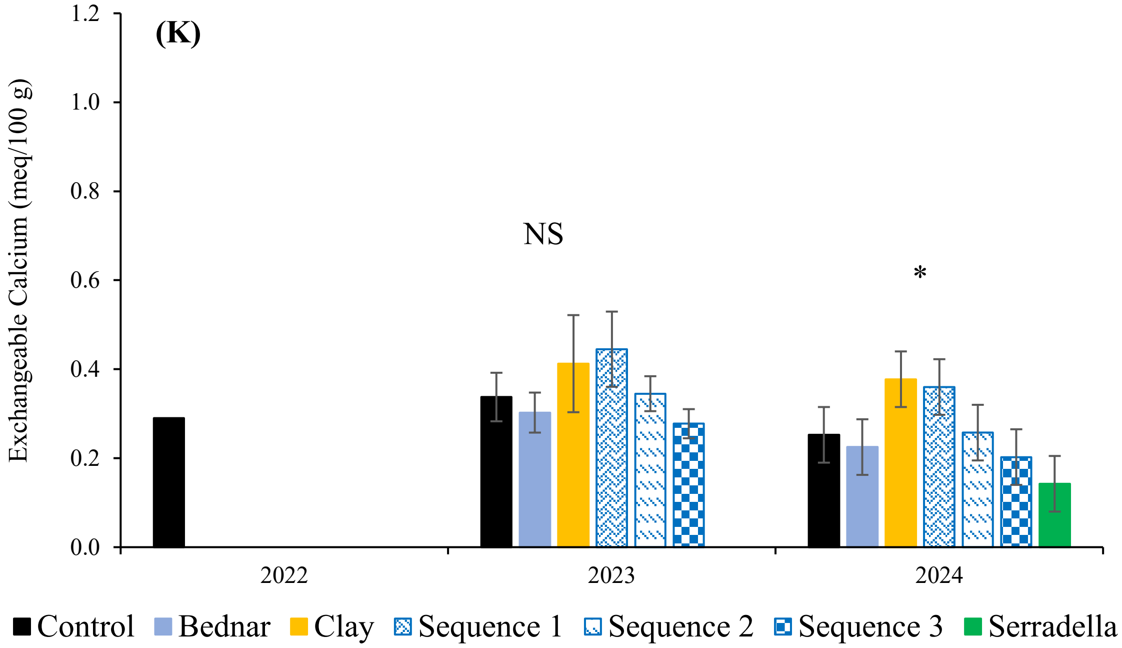
(H)
pH (CaCl2)
7
6
5
4
3
2
1
0
NS
*
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



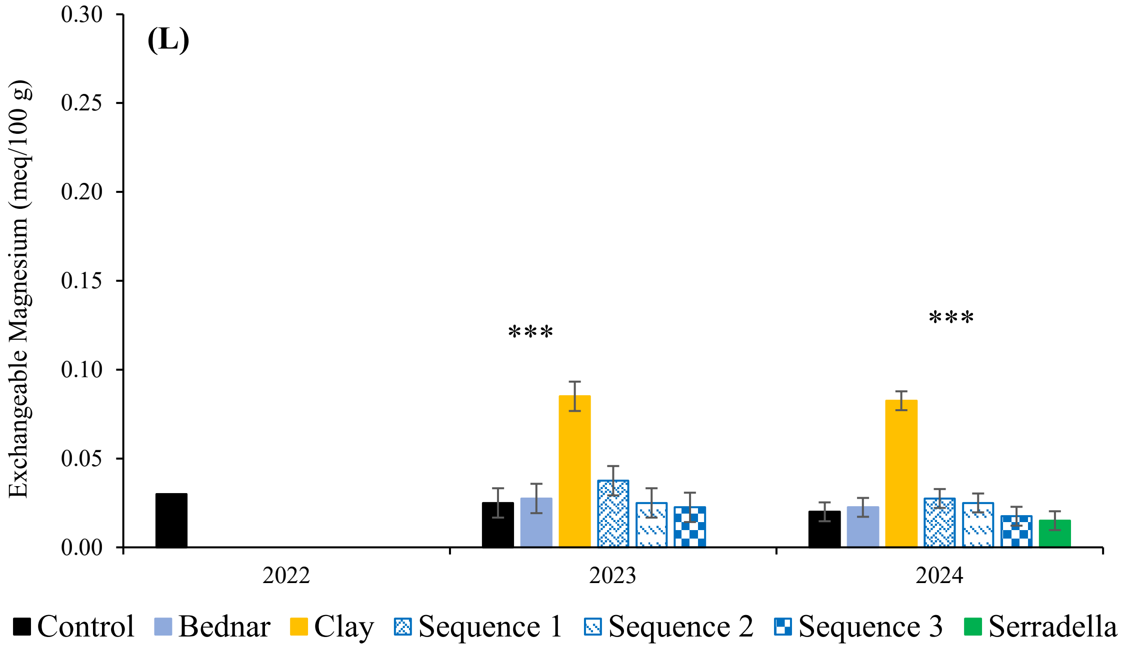
(I)
NS
**
pH (H_2O)
0
1
2
3
4
5
6
7
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella



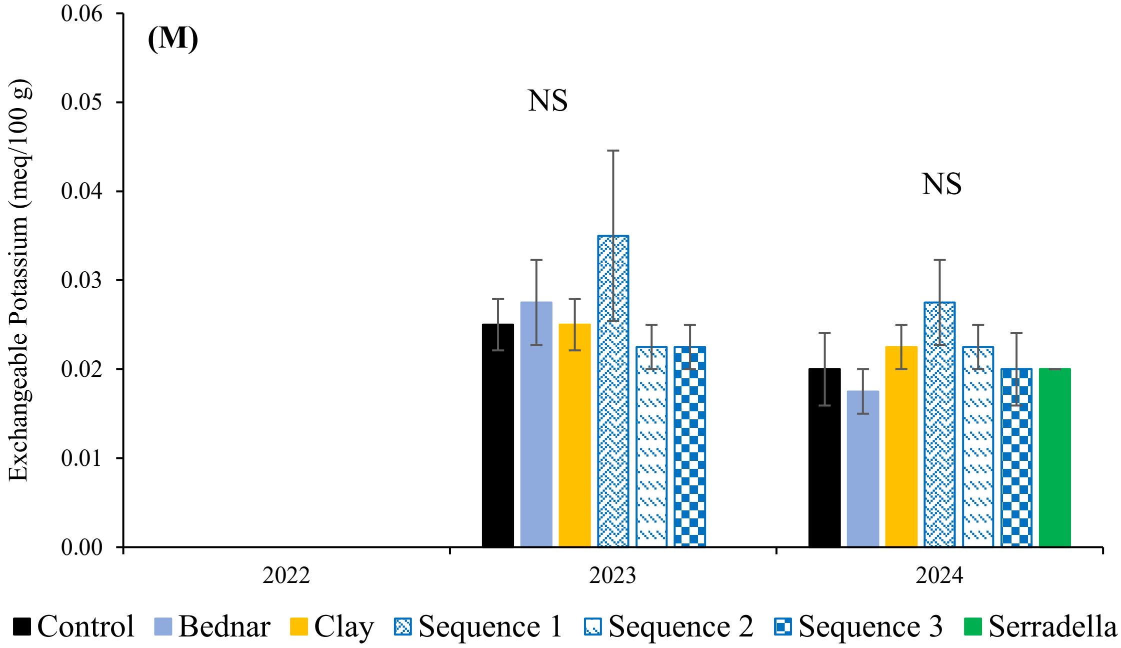
(J)
NS
NS
Exchangeable Aluminium (meq/100 g)
0.00
0.03
0.06
0.09
0.12
0.15
0.18
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

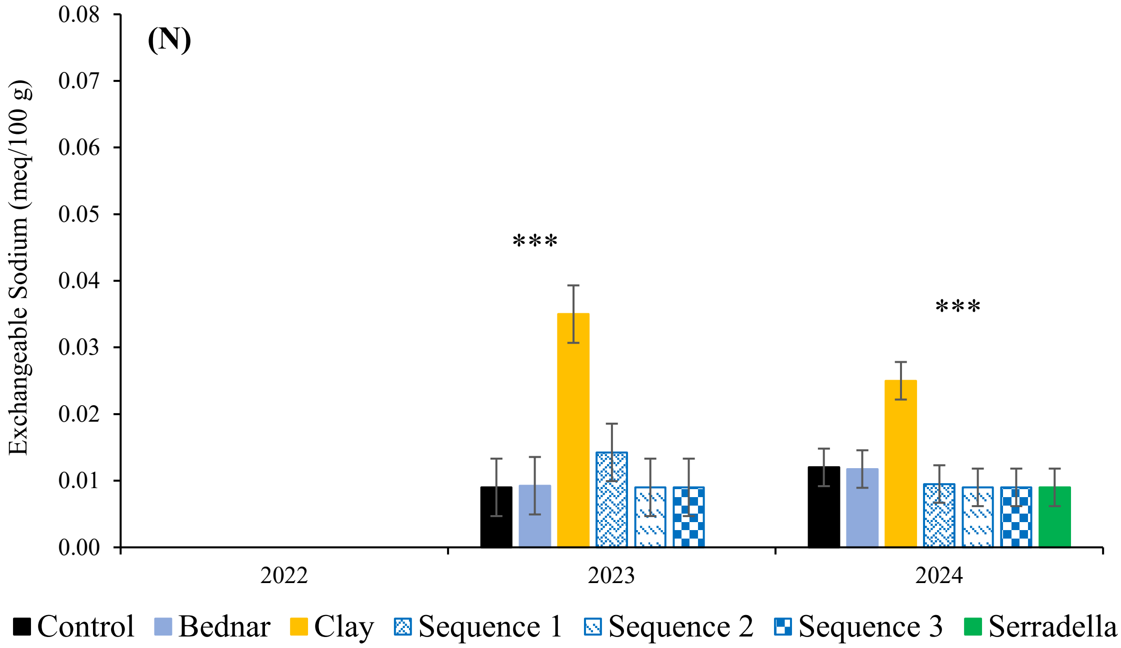


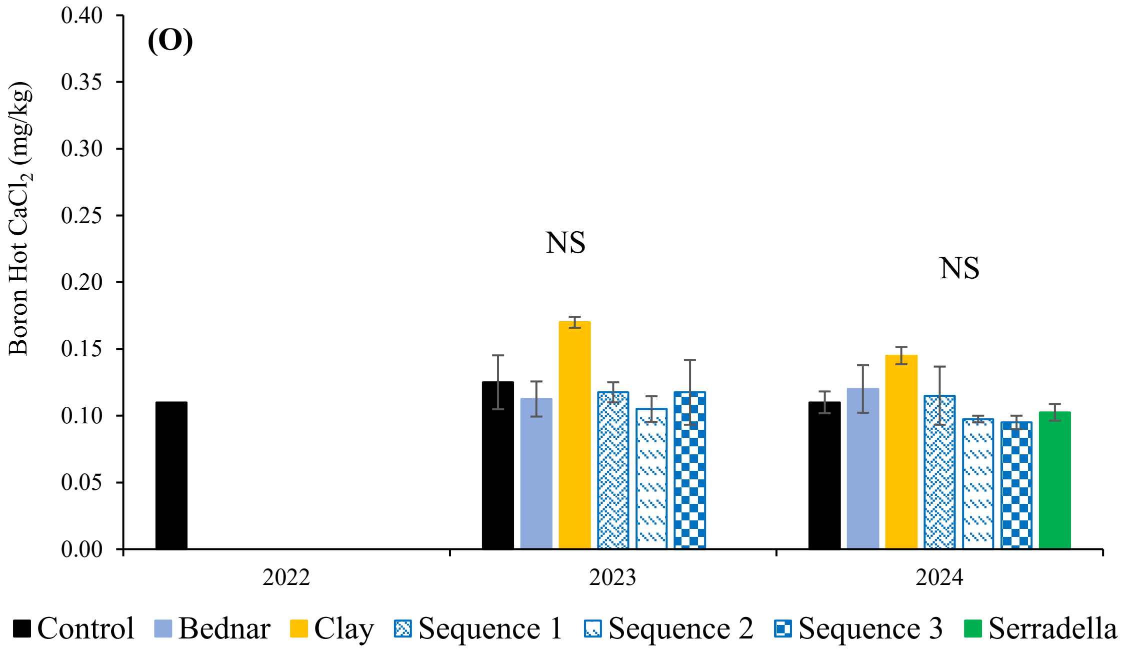
(K)
Exchangeable Calcium (meq/100 g)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NS
*
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

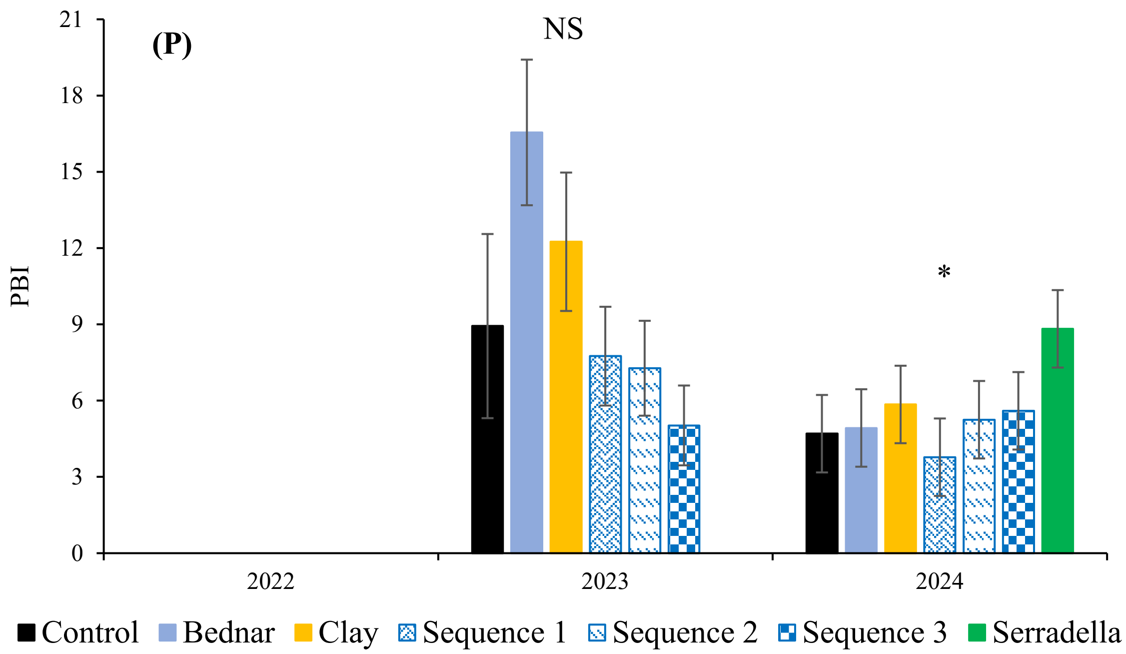


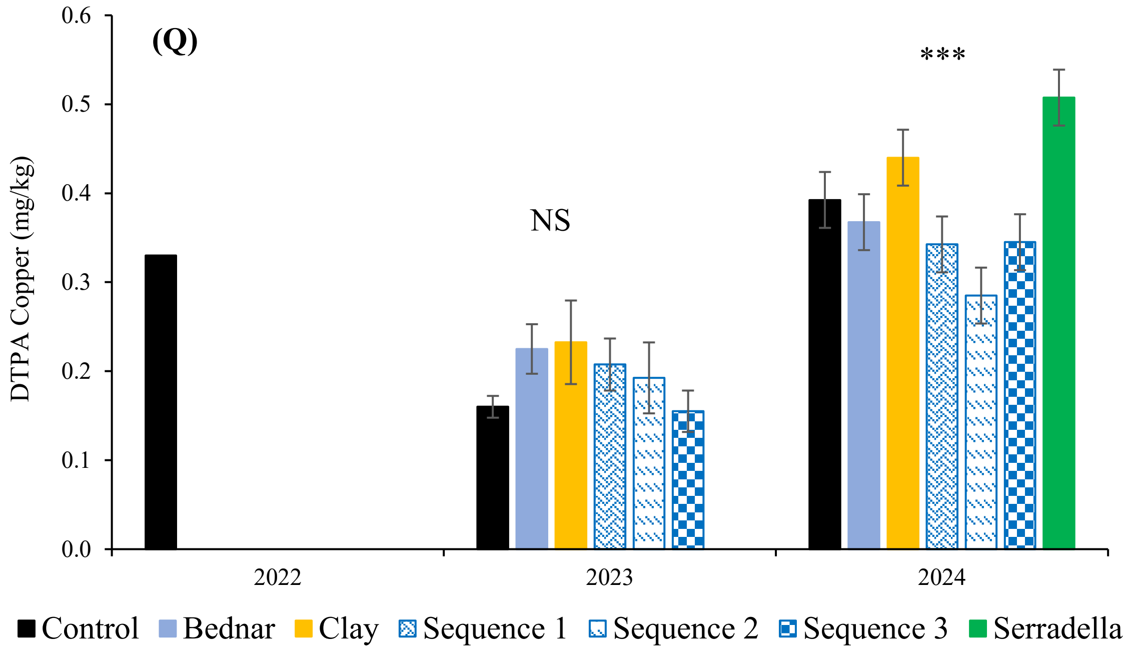
(L)
Exchangeable Magnesium (meq/100 g)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
***
***
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

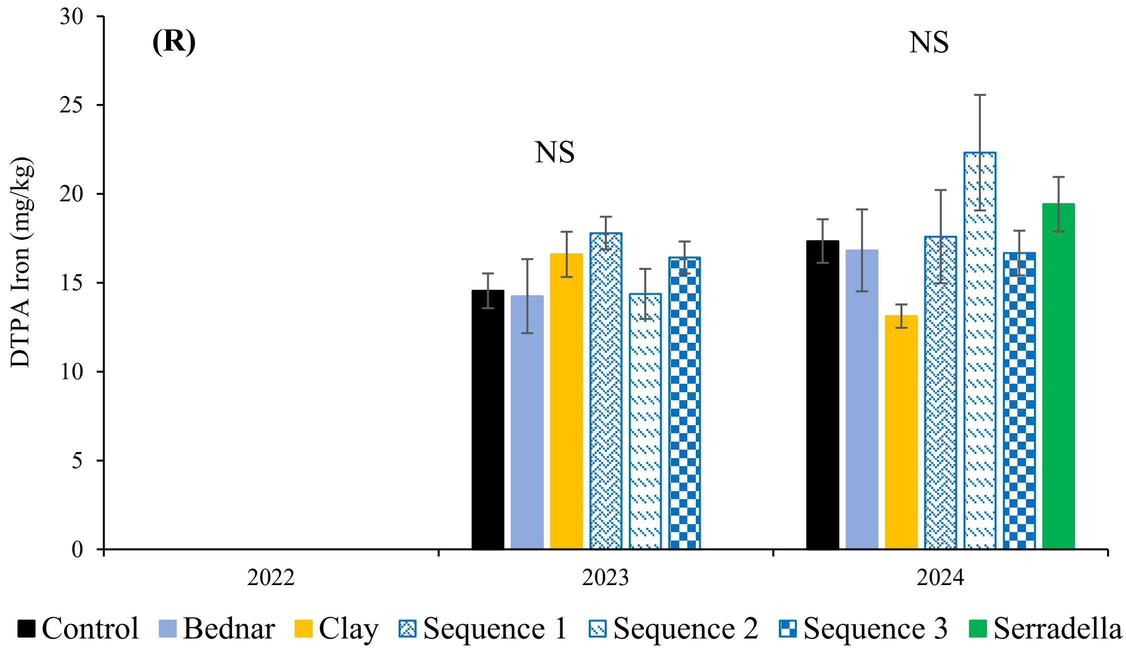

(M)
NS
NS
Exchangeable Potassium (meq/100 g)
0.06
0.05
0.04
0.03
0.02
0.01
0.00
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

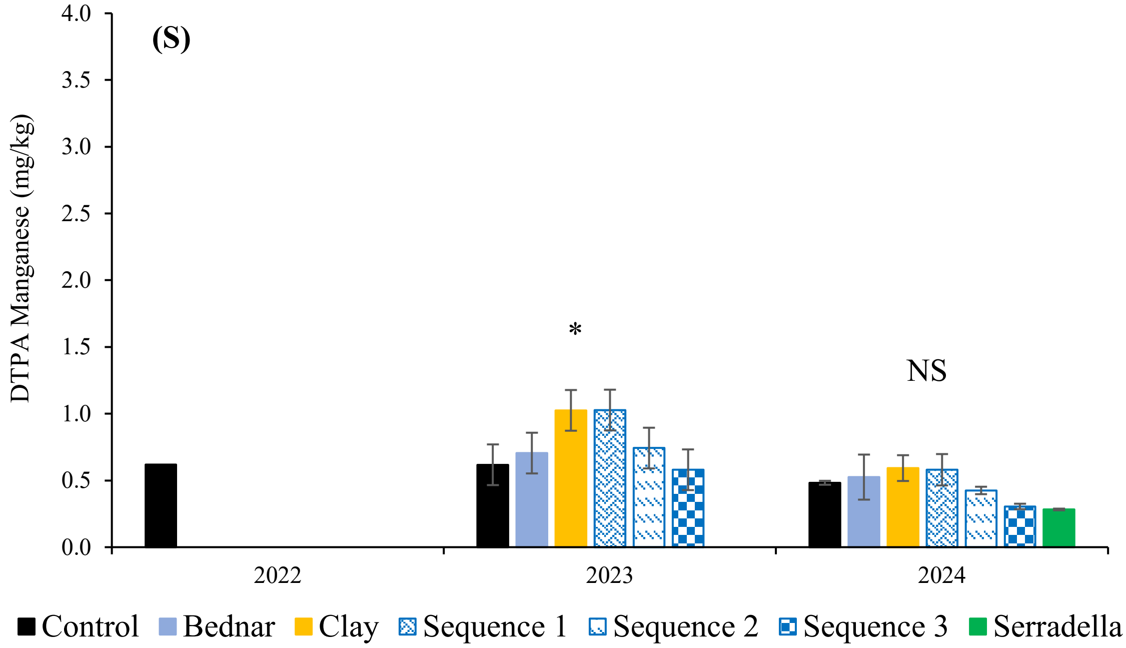

(N)
***
***
Exchangeable Sodium (meq/100 g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

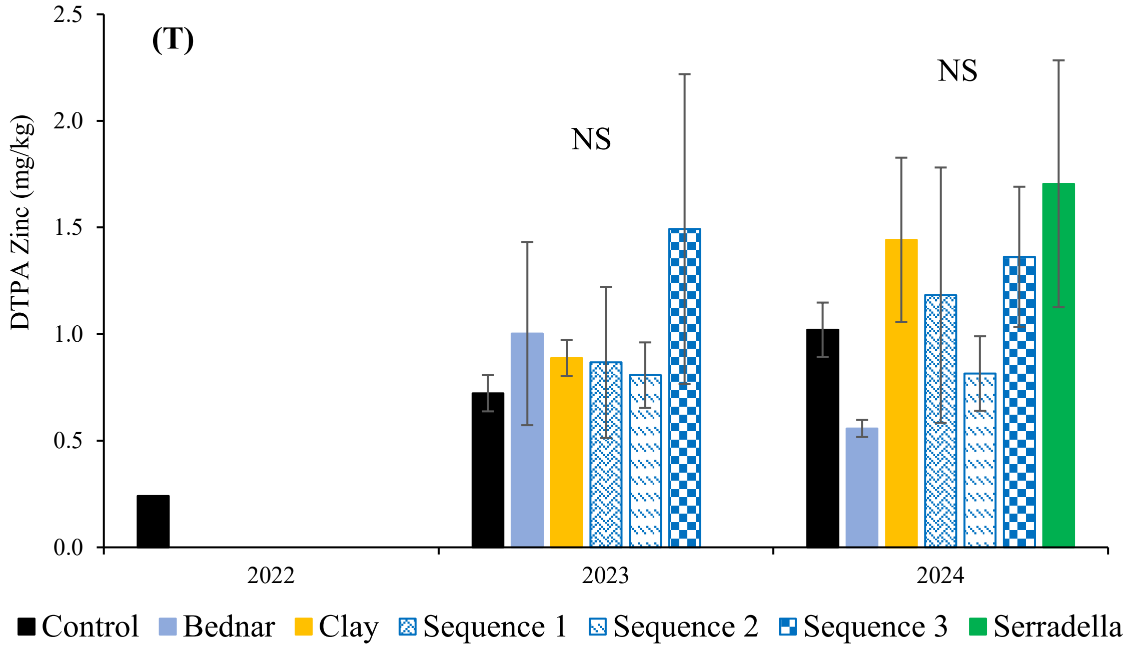

(O)
Boron Hot CaCl2 (mg/kg)
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(P)
PBI
NS
*
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(Q)
DTPA Copper (mg/kg)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
NS
***
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(R)
DTPA Iron (mg/kg)
0
5
10
15
20
25
30
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(S)
DTPA Manganese (mg/kg)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
*
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella


(T)
DTPA Zinc (mg/kg)
2.5
2.0
1.5
1.0
0.5
0.0
NS
NS
2022
2023
2024
Control
Bednar
Clay
Sequence 1
Sequence 2
Sequence 3
Serradella

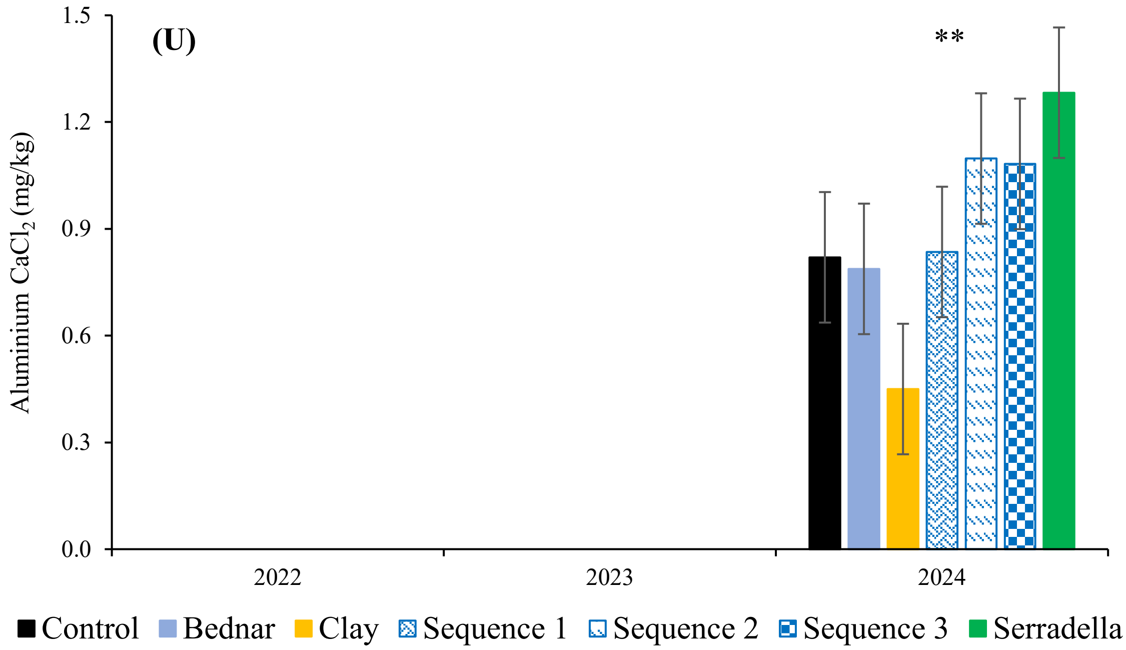


Fig. 10. Soil physio-chemical properties and nutrients status in 10-30 cm profile at Bullaring trial site, recorded at pre-seeding stage in 2024. **(A)** Ammonium Nitrogen (mg/kg). **(B)** Nitrate Nitrogen (mg/kg). **(C)** Phosphorus Colwell (mg/kg). **(D)** Potassium Kolwell (mg/kg). **(E)** Sulfur (mg/kg). **(F)** Organic Carbon (%). **(G)** Conductivity (dS/m). **(H)** pH ($CaCl_2$). **(I)** pH (H_2O). **(J)** Exchangeable Aluminium (meq/100 g). **(K)** Exchangeable Calcium (meq/100 g). **(L)** Exchangeable Magnesium (meq/100 g). **(M)** Exchangeable Potassium (meq/100 g). **(N)** Exchangeable Sodium (meq/100 g). **(O)** Boron hot $CaCl_2$ (mg/kg). **(P)** PBI. **(Q)** DTPA Copper (mg/kg). **(R)** DTPA Iron (mg/kg). **(S)** DTPA Manganese (mg/kg). **(T)** DTPA Zinc (mg/kg). **(U)** Aluminium $CaCl_2$ (mg/kg).

Data of each year have been statistically analysed independent of other years. NS = P > 0.1, † = P < 0.1, * = P < 0.05, ** = P < 0.01, *** = P < 0.001. n = 4. Where P is greater than 0.05, standard error of each individual mean has been inserted on each data bar as error bar. Where P is equal to or less than 0.05, a single standard error has been inserted as error bar on all the data bars for that particular year to compare the means of that year.

Sequence 1 was Oat+Vetch in 2022, Barley+Vetch in 2023 and Lupin+Vetch in 2024. Sequence 2 was Lupin+Vetch in 2022, Oat+Vetch in 2023 and Barley+Vetch in 2024. Sequence 3 was Barley+Vetch in 2022, Lupin+Vetch in 2023 and Oat+Vetch in 2024.

3.5. Key soil results (2022-2025)

Soil data recorded at pre-seeding in 2022-2024 have been presented above in **Section 3.4**. Here, the key soil data from 2025 is presented and compared with the previous years (Fig. 11-16).

3.5.1. Total Organic Carbon

Relative to 2022, the total organic carbon (TOC) in the untreated soil (Control) has slightly declined in the 0-10 and 10-30 cm profiles in 2025 but increased in the 30-45 cm profile (Fig. 11A-C).

In terms of the (TOC) in 2025, there are more variations between the treatments in the top 10 cm relative to the sub soil (10-45 cm). However, the variation between the replications in the top 10 cm has overshadowed the variations between the treatments which is why the treatments are not significantly different at 95% level of confidence but are different at 90% level of confidence. Comparison of the means of treatments (0-10 cm) at 90% level of significance shows that Bednar and Sequence 1 and 2 have sequestered greater C than control in the top 10 cm soil over the years (2022-2025) which is why Bednar has 1.9 t/ha more TOC while Sequence 1 and 2 have respectively 2.8 and 2.4 t/ha more TOC than control in the top 10 cm soil as measured in 2025 (Fig 11A).

In the 10-30 cm profile, the rest of the treatments were not different than control except Sequence 2 and 3 which had respectively 1.4 and 1.6 t/ha more TOC than control in 2025 (Fig 11B).

In 30-45 cm profile of the soil, all the treatments were statistically similar to control except clay which was found with significantly lower TOC than control in 2025 (Fig. 11C).

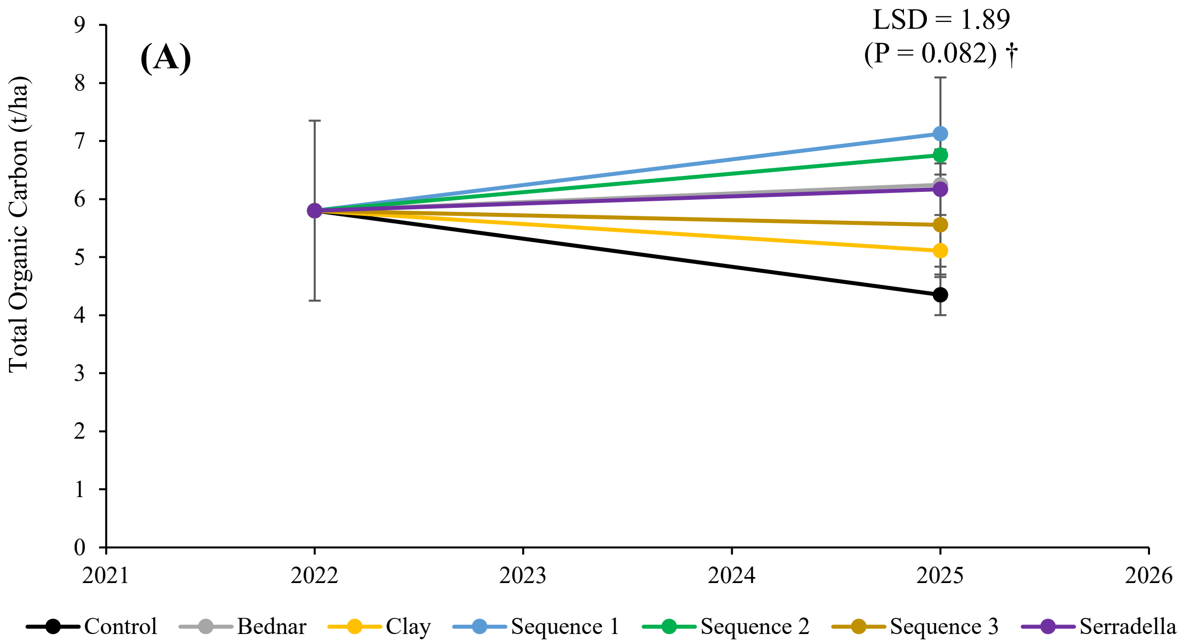
Crop sequences in general have increased the TOC between 2022 and 2025. Among the various sequences, Sequence 2 had more consistent effect on the TOC across the various soil depths despite producing smaller biomass during 2022-2024.

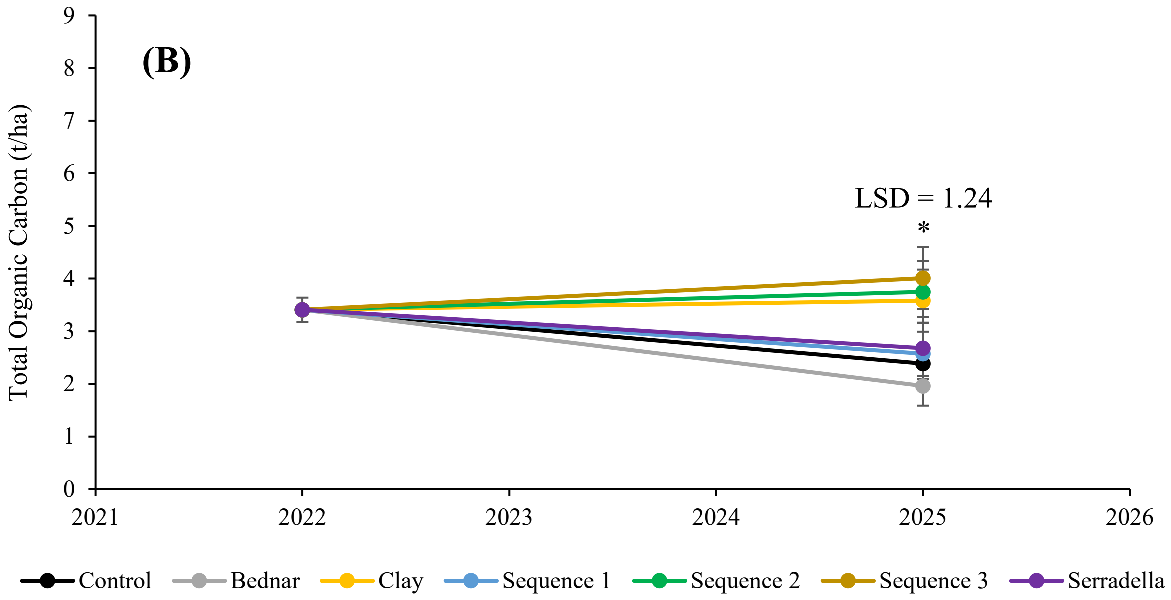
One can see that the crop sequences had vetch in common across the years and the only difference is the sequence of Oat, Barley and Lupin. In the Sequence 2, Lupin was sown in the first year followed by Oat and Barley in 2024 and 2025 respectively. While in the other 2 sequences, it was the other way around, i.e., the legume (Lupin) was sown later in the sequence.

Among the soil applied treatments, Bednar had only marginally increased the TOC in the top 10 cm profile while Clay was found with no to negative impact on the TOC across the profiles. Serradella also found with minimal to no effects on the TOC across the various soil profiles.

This implies that crop selection and their sequence over a period of 3-5 years may be more promising in the context of carbon sequestration than the soil manipulation and amendments.

Please note that Sequence 1 was Oat+Vetch in 2022, Barley+Vetch in 2023 and Lupin+Vetch in 2024. Sequence 2 was Lupin+Vetch in 2022, Oat+Vetch in 2023 and Barley+Vetch in 2024. Sequence 3 was Barley+Vetch in 2022, Lupin+Vetch in 2023 and Oat+Vetch in 2024.





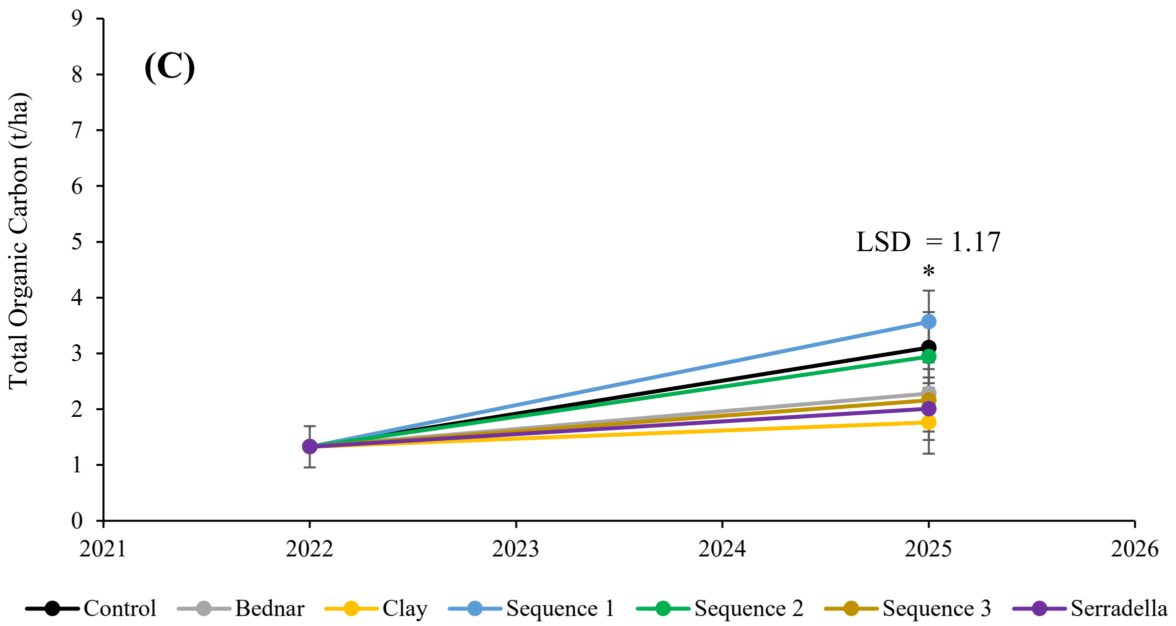


Fig. 11. Soil Total Organic Carbon in **(A)** 0-10 cm, **(B)** 10-30 cm and **(C)** 30-45 cm profile of various treatments at the Bullaring experimental site, recorded at pre-seeding in 2022 and 2025. The 2022 data is a baseline data recorded soon after the application of the treatments by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). The soil bulk density data had not been recorded in 2022, therefore, the 2025 bulk density data was used to calculate the total organic carbon per hectare for 2022.

NS = P > 0.1, † = P < 0.1, * = P < 0.05. Error bar is either the standard error of the treatment mean (n = 4) when P >0.05 or the standard error of the population mean across the treatments when P ≤ 0.05. Standard error of the population mean was calculated by using the following formula: Square Root of (2 × Mean Square of Error ÷ 4).

3.5.2. Soil nitrogen

$NH4^+$ did not differ between the treatments in the sub soil (10-30 cm) but differed in some of the observed years in the top soil (0-10 cm) (Fig. 12).

In the top soil, all the treatments had similar $NH4^+$ in 2023, however, they differed at 90% level of significance in 2024 and at 99% level of significance in 2025. In 2024, all the treatments had significantly lower $NH4^+$ than control except Bednar which was similar to the control (Fig. 12A). In 2025, only Sequence 1 had greater $NH4^+$ than control while the rest of the treatments were similar to control (Fig. 12A). Thus Sequence 1 had magnificently improved from 2024 to 2025 in terms of the $NH4^+$ in the top soil while a dip in the $NH4^+$ in the top soil could be observed from 2024 to 2025 in the top soil (Fig. 12A).

The nitrate nitrogen ($NO3^-$) was significantly different between the treatments in the top soil (0-10 cm) in 2023, 2024 and 2025 (Fig. 13). However, in the sub soil (10-30 cm), it only differed between the treatments in 2025 (Fig. 13).

In the top soil in 2023, treatments differed at 90% level of significance ($P = 0.54$). Only Sequence 2 differed than control, having 4 mg/g more nitrate N than control (Fig. 13A). In 2024, all the treatments were statistically similar to control except Sequence 1 which had significantly lower nitrate N than control in the top soil (Fig. 13A). Interestingly, in 2025, Sequence 1 had 8.5 mg/g greater nitrate N than control while the rest of the treatments were similar to control (Fig. 13A).

In the sub soil (10-30 cm), only Sequence 1 had greater nitrate N than control while the rest of the treatments were similar to control (Fig. 13B).

Thus Sequence 1 magnificently improved between 2024 and 2025 both in terms of the nitrate as well as ammonium N (Fig. 12-13), which could be attributed to the inclusion of legume in Sequence 1 (Vetch + Lupin) in 2024.

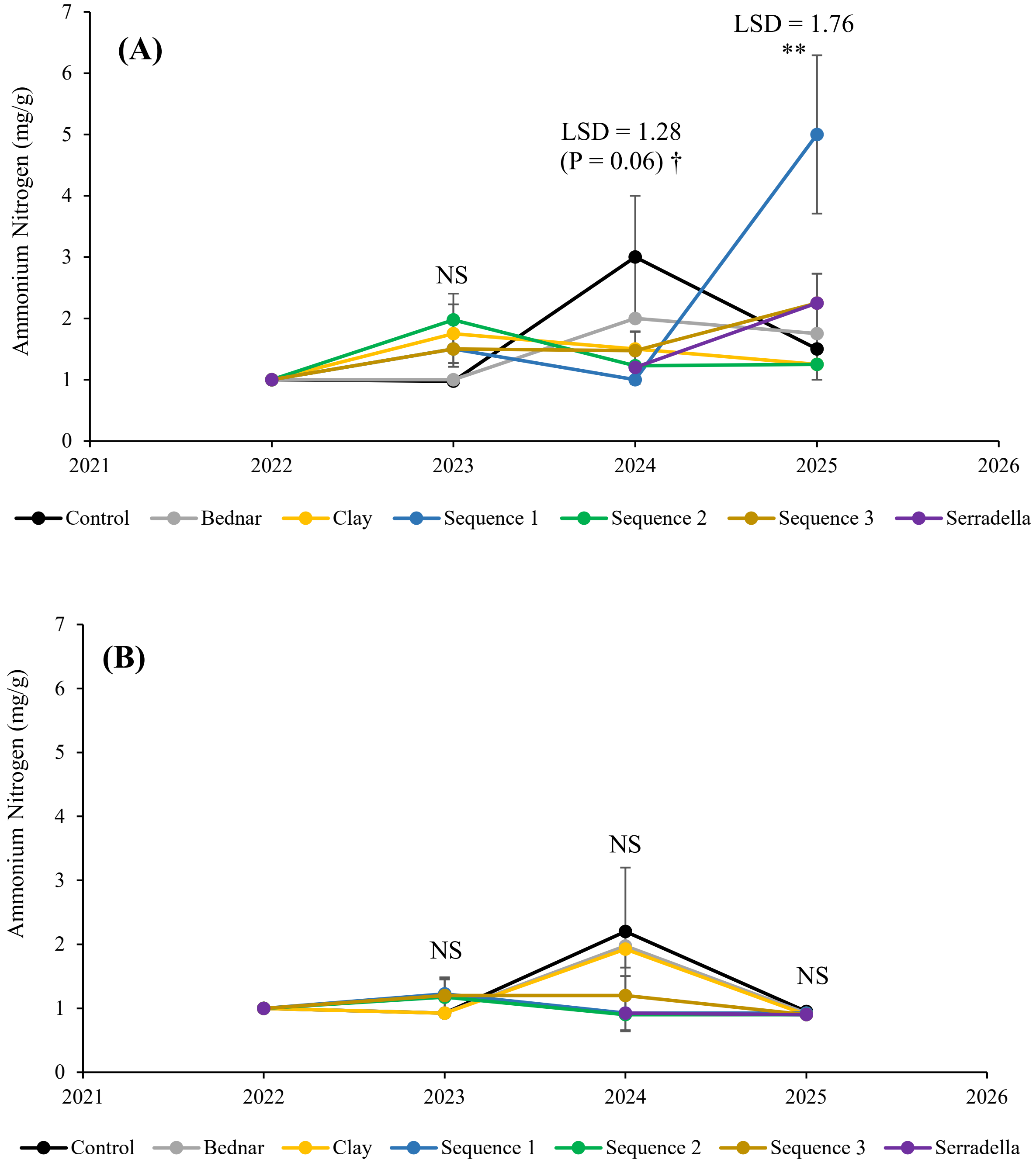
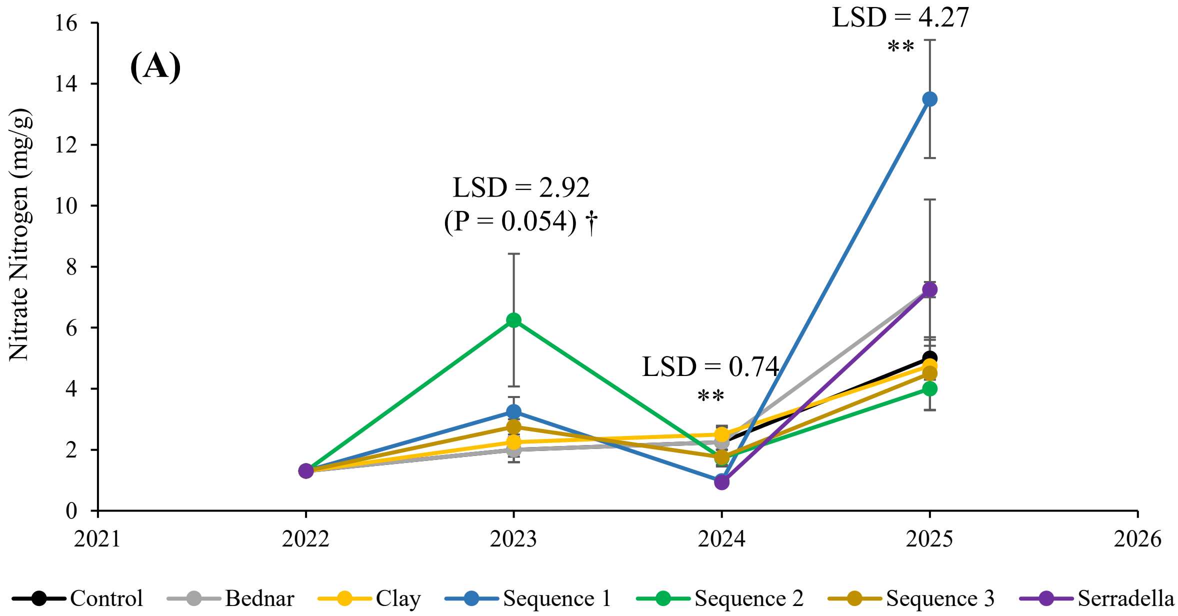


Fig. 12. Ammonium nitrogen in **(A)** 0-10 cm and **(B)** 10-30 cm profile of various treatments at the Bullaring experimental site, recorded at pre-seeding in 2022-2025. The 2022 data is a baseline data recorded soon after the application of the treatments by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). In 2023, all the treatments were sampled except Serradella, while samples were collected from all the treatments in 2024 and 2025.

NS = P > 0.1, † = P < 0.1, * = P < 0.05. Error bar is the standard error of the treatment mean (n = 4). LSD value for the comarison of means is given where treatments were significantly different at least at 90% level fo significance.



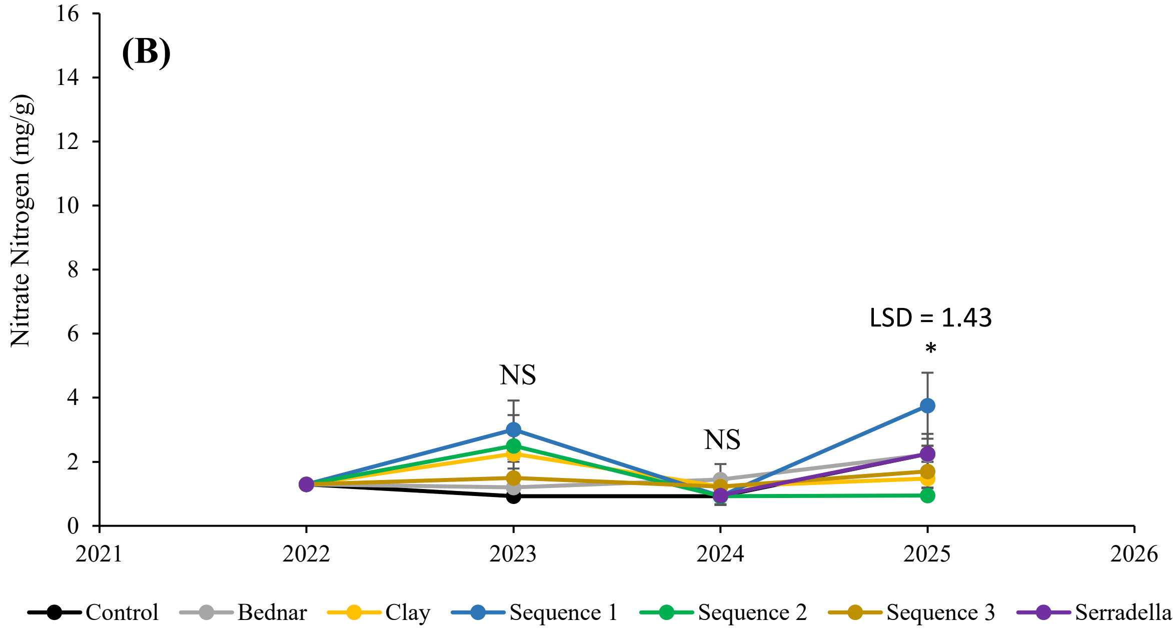


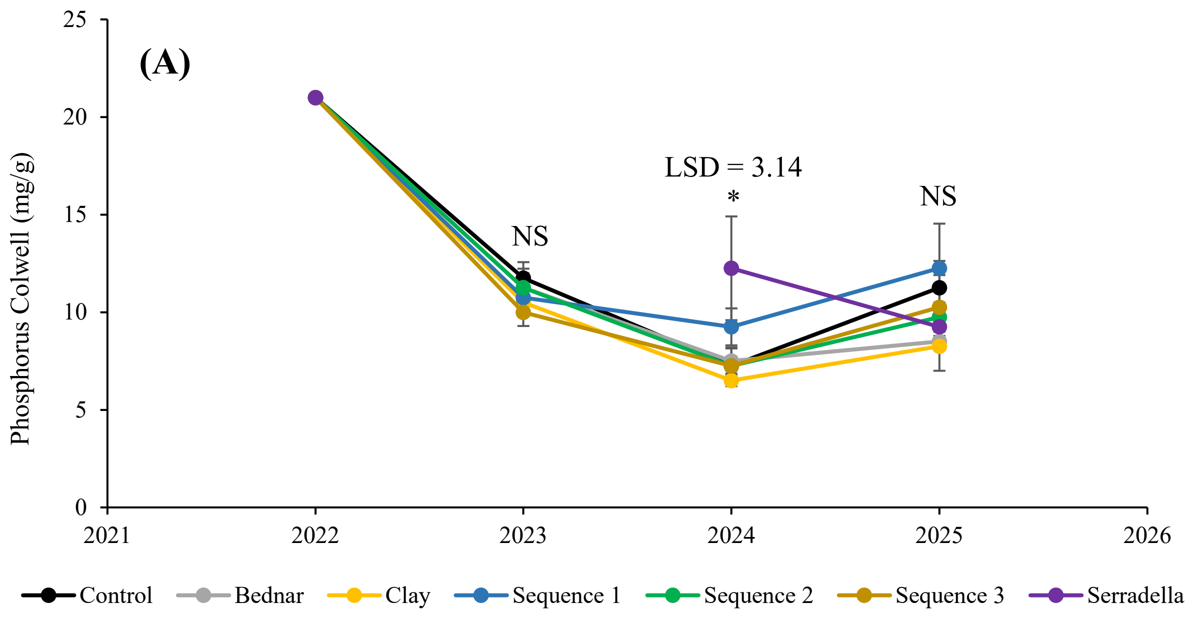
Fig. 13. Nitrate nitrogen in **(A)** 0-10 cm and **(B)** 10-30 cm profile of various treatments at the Bullaring experimental site, recorded at pre-seeding in 2022-2025. The 2022 data is a baseline data recorded soon after the application of the treatments by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). In 2023, all the treatments were sampled except Serradella, while samples were collected from all the treatments in 2024 and 2025.

NS = P > 0.1, † = P < 0.1, * = P < 0.05. Error bar is the standard error of the treatment mean (n = 4). LSD value for the comarison of means is given where treatments were significantly different at least at 90% level fo significance.

3.5.3. Phosphorus (P)

Phosphorus was significantly different between the treatments in 2024 both in the top soil (0-10 cm) as well as sub soil (10-30 cm) but did not differ between the treatments in 2023 and 2025 (Fig. 14).

Both in the top as well as sub soil in 2024, only serradella was significantly different than control with about 5 mg/g more P than control in the top soil as well as in the sub soil (Fig. 14A-B). Serradella has been previously reported to have a positive effect on the soil P. For instance, Vitow et al. (2021) reported that the Serradella planted plots with no P fertiliser application showed higher P values than the bare fallow subplots. This could be partly because of lower P uptake by Serradella compared to the grain crops such as Oat (Moir et al., 2014). A drop in the P in Serradella treatment from 2024 to 2025 may be because of high weed infestation serradella plots. Capeweed was particularly found in abundance in the Serradella plots.



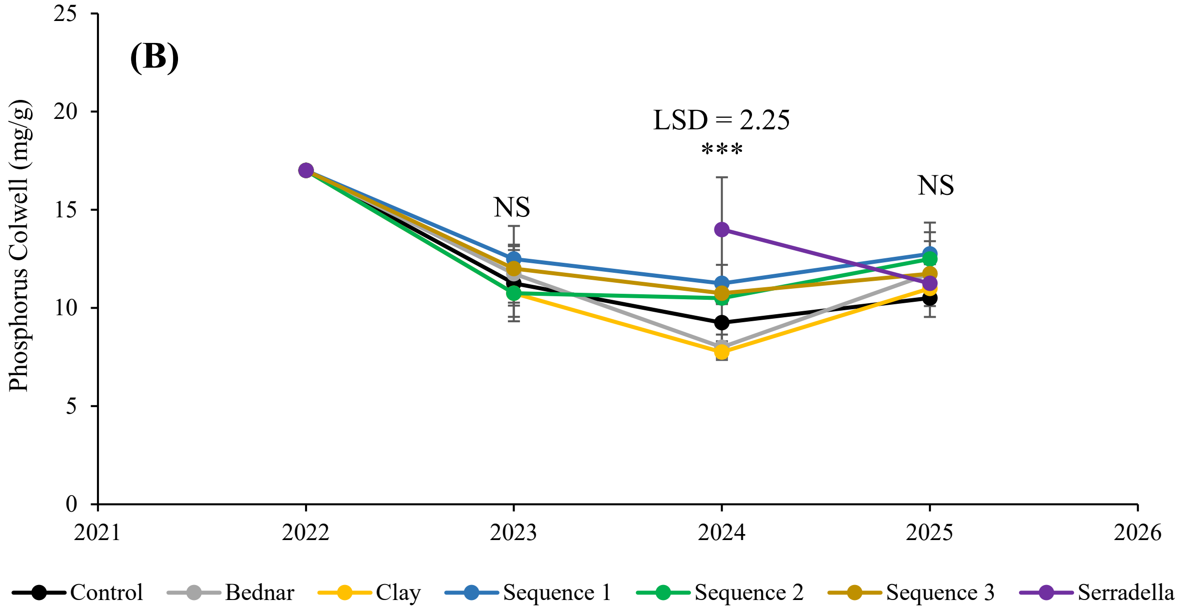
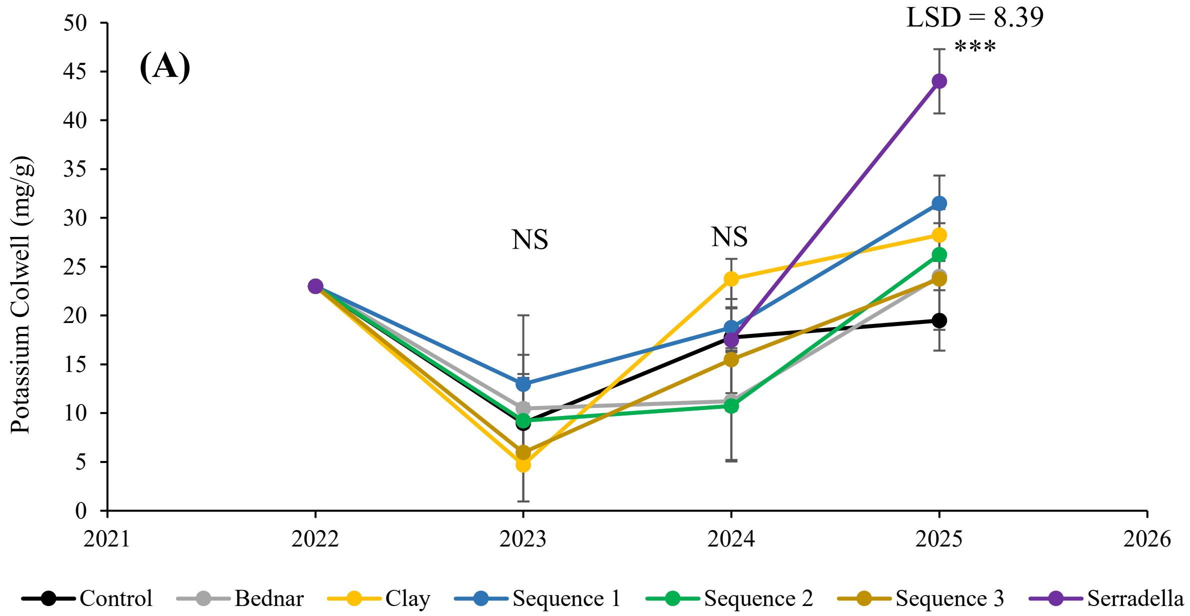


Fig. 14. Phosphorus colwell in **(A)** 0-10 cm and **(B)** 10-30 cm profile of various treatments at the Bullaring experimental site, recorded at pre-seeding in 2022-2025. The 2022 data is a baseline data recorded soon after the application of the treatments by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). In 2023, all the treatments were sampled except Serradella, while samples were collected from all the treatments in 2024 and 2025.

NS = P > 0.1, † = P < 0.1, * = P < 0.05. Error bar is the standard error of the treatment mean (n = 4). LSD value for the comarison of means is given where treatments were significantly different at least at 90% level fo significance.

3.5.4. Potassium (K)

Potassium largely remained similar between the treatments across the years and soil profiles except in the top soil in 2025 (Fig. 15). Clay, Sequence 1 and Serradella had significantly greater K than control in the top soil in 2025 (Fig. 15A). Among the mentioned three treatments, Serradella sown soil was found with the highest K content of 44 mg/g in the top 10 cm followed by Sequence 1 (Fig. 15A). This might be attributed to smaller biomass produced by serradella compared to other treatments in 2024 (Fig. 7A-B) as well as efficient use of the soil K by Serradella as reported by Brennan and Bolland (2006).



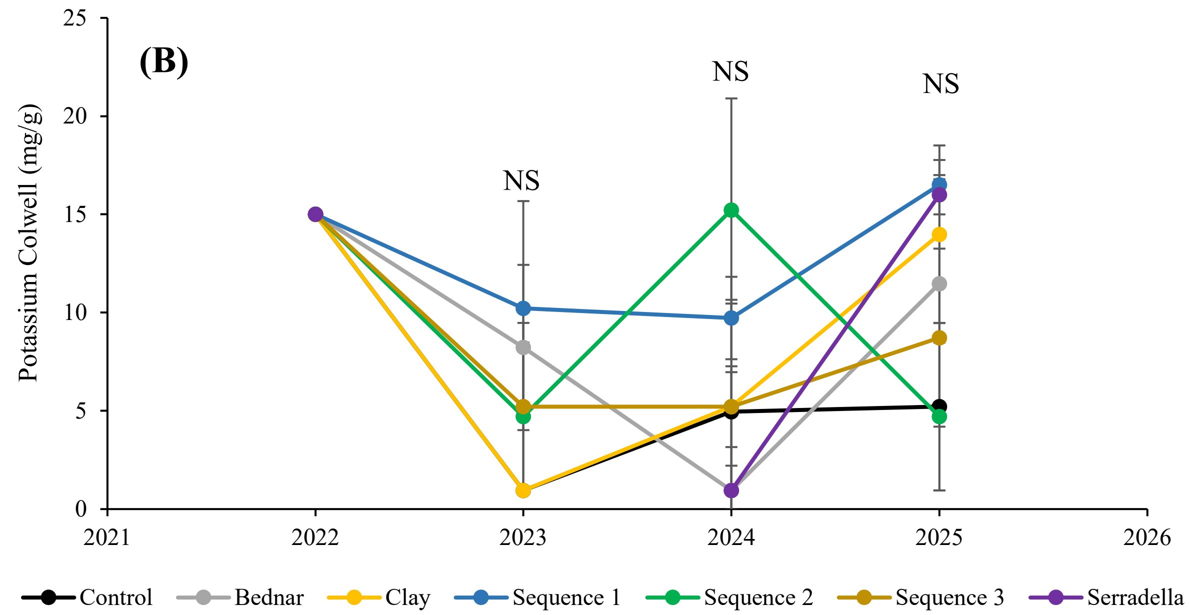
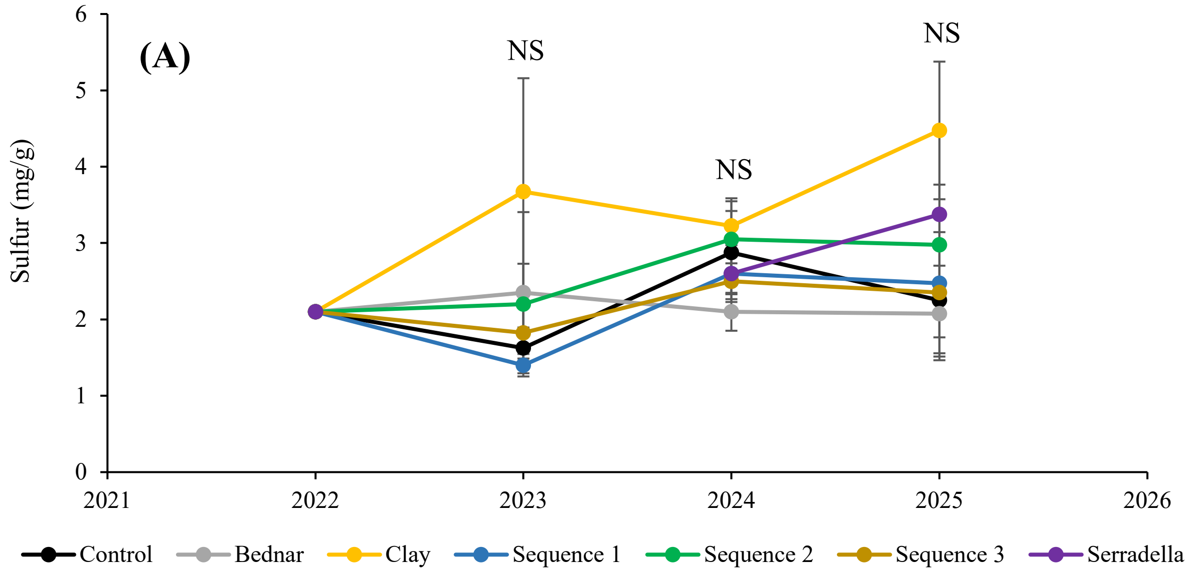


Fig. 15. Potassium colwell in **(A)** 0-10 cm and **(B)** 10-30 cm profile of various treatments at the Bullaring experimental site, recorded at pre-seeding in 2022-2025. The 2022 data is a baseline data recorded soon after the application of the treatments by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). In 2023, all the treatments were sampled except Serradella, while samples were collected from all the treatments in 2024 and 2025.

NS = P > 0.1, † = P < 0.1, * = P < 0.05. Error bar is the standard error of the treatment mean (n = 4). LSD value for the comarison of means is given where treatments were significantly different at least at 90% level fo significance.

3.5.5. Sulfur (S)

None of the studied treatment could significantly alter the level of Sulfur in the soil relative to control (Fig. 16).



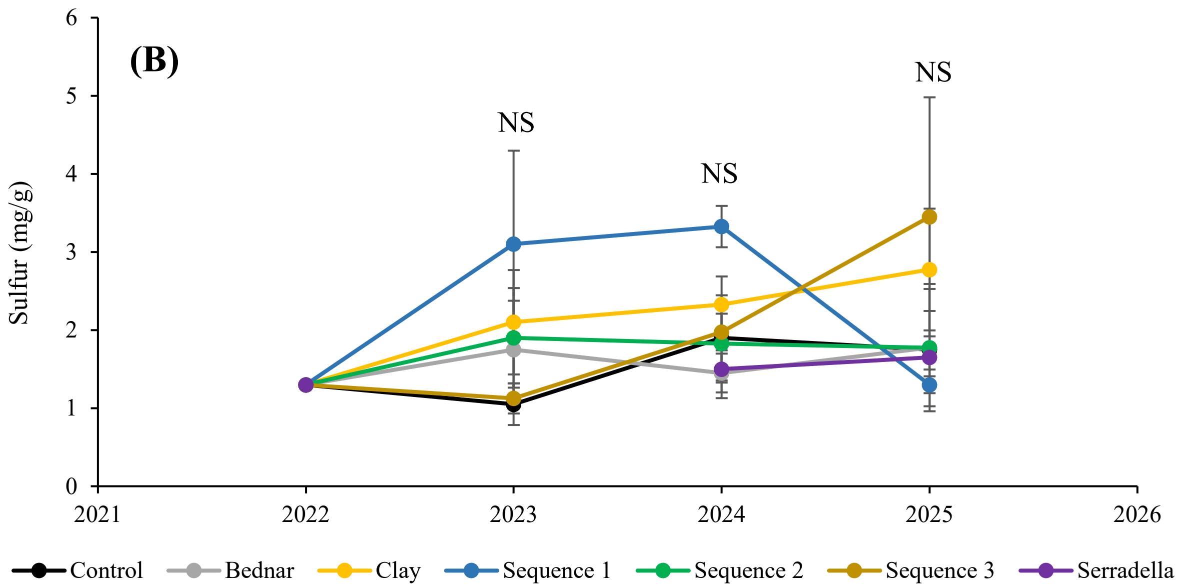


Fig. 16. Sulfur in **(A)** 0-10 cm and **(B)** 10-30 cm profile of various treatments at the Bullaring experimental site, recorded at pre-seeding in 2022-2025. The 2022 data is a baseline data recorded soon after the application of the treatments by taking one composite sample per replicate of the control, each sample from 4 different sampling points within the plot (total 4 composite samples from 4 replicates of control). In 2023, all the treatments were sampled except Serradella, while samples were collected from all the treatments in 2024 and 2025.

NS = P > 0.1, † = P < 0.1, * = P < 0.05. Error bar is the standard error of the treatment mean (n = 4). LSD value for the comarison of means is given where treatments were significantly different at least at 90% level fo significance.

Conclusion

It could be inferred from the results of the current study that plant-based treatments could be more promising for the highly sandy soil such as Bullaring, as most of the nutrients in the soil were improved by the plant-based treatments while the soil-based treatments could produce little-to-no effects. Though, clay improved soil nutrients and other chemical properties in 2024, but that improvement did not reflect in the crop, possibly because of low and untimely rainfall.

Keeping in view the improved soil properties in the plant-based treatments, all the treatments were seeded with the same crop (Canola) in 2025 to see how those improvements would reflect in the main crop.

References

Angus J., Kirkegaard J., Hunt J., Ryan M., Ohlander L., Peoples M. (2015) Break crops and rotations for wheat. Crop and pasture science 66:523-552.

Brennan R., Bolland M. (2006) Comparing the potassium requirements of five pasture species. Australian Journal of Experimental Agriculture 46:659-667.

Bybee-Finley K.A., Ryan M.R. (2018) Advancing intercropping research and practices in industrialized agricultural landscapes. Agriculture 8:80.

Godde C.M., Thorburn P.J., Biggs J.S., Meier E.A. (2016) Understanding the impacts of soil, climate, and farming practices on soil organic carbon sequestration: a simulation study in Australia. Frontiers in plant science 7:661.

Hall D., Bell R., Sochacki S., Davies S. (2015) Long term effects of claying on non-wetting and plant nutrition. Agribusiness crop updates, Perth'.(Department of Agriculture and Food/Grains Research and Development Corporation: Perth, Western Australia).

Hall D.J., Davies S.L., Bell R.W., Edwards T.J. (2020) Soil management systems to overcome multiple constraints for dryland crops on deep sands in a water limited environment on the south coast of Western Australia. Agronomy 10:1881.

Hall D.J., Jones H., Crabtree W., Daniels T. (2010) Claying and deep ripping can increase crop yields and profits on water repellent sands with marginal fertility in southern Western Australia. Soil Research 48:178-187.

Harries M., Flower K.C., Scanlan C.A., Rose M.T., Renton M. (2020) Interactions between crop sequences, weed populations and herbicide use in Western Australian broadacre farms: findings of a six-year survey. Crop and pasture science 71:491-505.

Isbell F., Adler P.R., Eisenhauer N., Fornara D., Kimmel K., Kremen C., Letourneau D.K., Liebman M., Polley H.W., Quijas S. (2017) Benefits of increasing plant diversity in sustainable agroecosystems. Journal of ecology 105:871-879.

Moir J., Schwass M.J., Moot D. (2014) Lime, phosphorus and sulphur response of French serradella (Ornithopus sativus) grown in an acid upland soil.

Nazir M.J., Li G., Nazir M.M., Zulfiqar F., Siddique K.H., Iqbal B., Du D. (2024) Harnessing soil carbon sequestration to address climate change challenges in agriculture. Soil and Tillage Research 237:105959.

Roesch-McNally G.E., Arbuckle J., Tyndall J.C. (2018) Barriers to implementing climate resilient agricultural strategies: The case of crop diversification in the US Corn Belt. Global environmental change 48:206-215.

Sharma G., Shrestha S., Kunwar S., Tseng T.-M. (2021) Crop diversification for improved weed management: A review. Agriculture 11:461.

Vitow N., Zicker T., Chiba A., Zacher A., Eichler-Löbermann B., Schulz S., Schloter M., Baum C., Leinweber P. (2021) Impact of the legume catch crop serradella on subsequent growth and p mobilization under barley in different fertilization treatments. Agronomy 11:2437.