

Deliverable D5.2: Agronomic behaviour in a wide range of conditions (Task 5.4 Ecotron trial)



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Document Summary

Deliverable D5.2: Agronomic behaviour in a wide range of conditions (Report on Task 5.4 Ecotron trial)

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Author(s): Jennifer Michel (ULiège)

Contributor(s): Lucas Bergenhuizen (ULiège)

Reviewer(s): Astrid Solvåg Nesse (NBIO), Miriam Pinto (NEIKER)

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Abstract

The Ecotron trial evaluated the agronomic performance and the environmental impact of four bio-based fertilisers (BBFs) compared to a synthetic fertilizer (SYN) under a historic reference and a future RCP8.5 climate scenario.

While SYN fertilization resulted in slightly higher crop biomass production compared to fertilization with BBFs in the reference climate, all BBFs reached similar marketable yields for both broccoli (head fresh weight) and spinach (leaf fresh weight). The future climate negatively impacted crop growth irrespective of the fertiliser used, with the largest decrease in marketable yield observed under BBF2 (CAT1) and SYN.

CO₂-emissions were not significantly different between the fertiliser treatments or the climates. N₂O-emissions were significantly higher in systems fertilised with BBFs compared to those under SYN or no fertilisation, probably linked to enhanced soil microbial activity under BBFs (estimated via hydrolysis of fluorescein diacetate).

There were no statistically significant differences in nutrient exports (C, N, P) in aboveground biomass per area for neither of the fertilisers nor per climate, with inconsistent trends for the different fertiliser x climate modalities, highlighting the complexities of nutrient dynamics in cropping systems under environmental change.

Overall, these results support BBFs as viable agronomic alternatives to SYN, which can reach similar yields and products of similar nutritious quality in terms of N and P contents. However, further research is needed to address climate-induced yield penalties observed for all fertilizers (BBFs & SYN) and possibly address enhanced N₂O-emissions as observed for systems fertilised with BBFs.

All environmental and agronomic data generated during the Ecotron trial has been delivered to task 5.3 to test the predictions of the DNDC model, which has been parametrized based on the data from field trials (task 5.2).

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Glossary

BBCH: Scale to numerically classify the phenological development stages of plants

BBF: Bio-based fertiliser

CER: Controlled environment room

DNDC: DeNitrification-DeComposition model

FDA: Fluorescein diacetate

GHG: Greenhouse gas

NBI: Nitrogen balance index

NO: No fertiliser used

NUE: Nitrogen use efficiency

PUE: Phosphorous use efficiency

RCP8.5: Representative Concentration Pathway 8.5 W m⁻²

SYN: Synthetic fertiliser



1 Experimental set-up of Ecotron trial (Task 5.4)

The Ecotron trial (task 5.4) evaluated the agronomic performance and environmental impact of different BBFs compared to a synthetic fertilizer (SYN) under a historic reference and a future RCP8.5 climate scenario. In total, four BBFs were tested on two crops (broccoli and spinach).

1.1 Climate scenarios

Two climate scenarios were generated to evaluate the performance and climate sensitivity of BBFs under realistic growing conditions: a historic reference climate (1981-2017) providing favourable growing conditions for the crops to investigate the maximum potential of the different BBFs, and a projected future climate scenario (2095) with higher atmospheric CO₂-concentrations and higher ambient temperature to investigate the effectiveness of the different BBFs in the cropping systems under climate change. The cumulative precipitation in the future climate scenario was similar to the historic reference climate, but comprised periods of both more intense rainfall and prolonged droughts, which is in line with most climate projections forecasting increased frequency of extreme weather events in Europe and beyond (IPCC, 2021; Fischer et al. 2013). The historic reference climate consisted of the smoothed mean of the environmental parameters of the reference period 1981-2017 to provide non-stressing plant growth conditions throughout the study period and provide a representative climate without any year-specific climate anomalies.

The smoothed mean of historical climate from 1981 to 2017 was generated on the basis of continuous weather data collected in central Belgium at the Ernage weather station (50°35'30.6"N, 4°40'19.9"E). Based on these historic records, the future climate for this region was predicted for the period 2070-2100 using the Alaro-0 model (Giot et al., 2016) and the RCP8.5 W m⁻² scenario (IPCC, 2014). The year 2095 was chosen as it represents a common future scenario with increased temperature (+2,5°C) and atmospheric CO₂ levels (+360 ppm), and more intense distribution of rain and drought events (Figure 1). The climate scenarios are implemented at a high temporal resolution in the TERRA-Ecotron, where environmental parameter are adjusted every five minutes to provide realistic scenarios that mimic diurnal and seasonal climate variability.



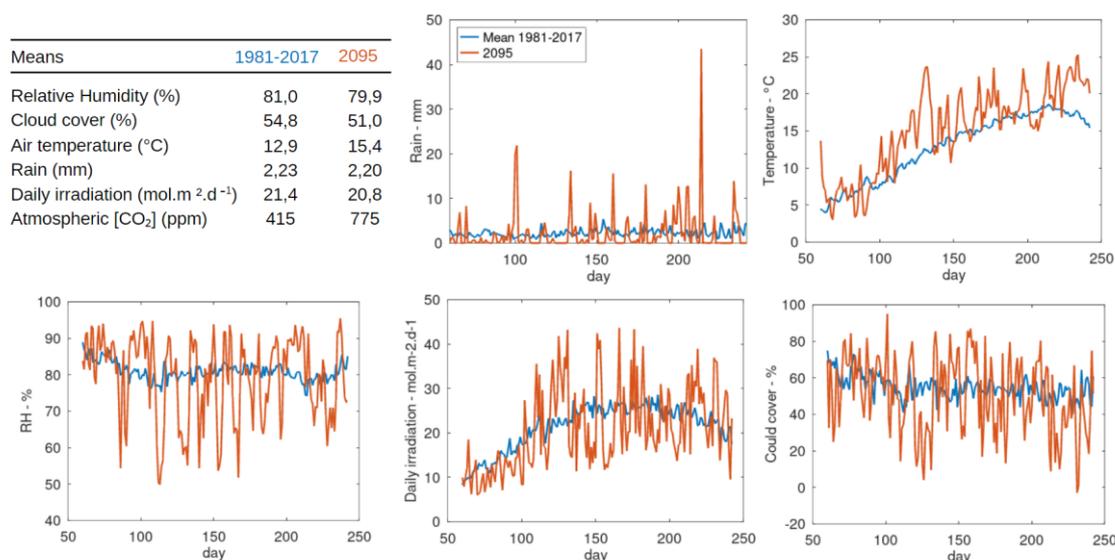


Figure 1: Main environmental parameter for the two climate scenarios 1981-2017 in blue and future scenario 2095 in orange.

1.2 Representative soil type

An arable field was identified in central Belgium (50°33'54.8104"N, 4°42'9.9349"E) with well documented farming history and soil conditions representative of central Europe, but a low background of synthetic or organic inputs. The studied field previously had a 2-year succession of textile hemp (*Cannabis sativa* subsp. *Sativa*, 2022 and 2023), preceded by two years of meadow (mix of *Lolium perenne*, *Agrostis stolonifera*, *Alopecurus pratensis*, *Phleum pratense*, *Agrostis capillaris*, *Ranunculus acris*, *Trifolium pratense*, *Lotus corniculatus*, 2020 and 2021), and preceded by maize (*Zea mays*, 2019). No fertilisers or plant protection products were used on hemp or grassland, but nitrogenous fertiliser was applied for maize cultivation. The soil is classified as clayey loam and has a humus content of 3.4% and a C:N of 13, with 17 g kg⁻¹ organic carbon and 1.3 g kg⁻¹ total nitrogen (Table 1). For the Ecotron experiment, a total of n=54 intact and undisturbed soil monoliths with a dimension of 50x50x50cm (125l) each were sampled in April 2024 (Figure 2).

Table 1: Soil characterisation

Humus (%)	Organic carbon (g kg ⁻¹)	Carbonate (%)	Total nitrogen (g kg ⁻¹)	C:N	N-NO ₃ (g kg ⁻¹)	N-NH ₄ (g kg ⁻¹)	P-PO ₄ (g kg ⁻¹)
3.383 ± 0.508	17.000 ± 2.757	1.200 ± 0.49	1.300 ± 0.00	13.077 ± 2.121	3.974 ± 0.465	0.384 ± 0.237	0.085 ± 0.002



Figure 2: Sampling of soil monoliths in the field and set-up in the Ecotron. After excavating two trenches, cubes are cut to exact size (50x50x50cm) by hand (A) and metal sheets are placed around them (B). Cubes are then closed at the top, flipped, the bottom is straightened and a 1.5 cm layer of gravel added to maintain drainage. Cubes are then closed, flipped again and reopened at the top. Thereafter cubes are transported to the Ecotron (B) and placed onto plateaus to be moved into the CERs (C).

1.3 Fertilisers tested

Four bio-based fertilisers (BBFs) and a synthetic fertiliser (SYN) were tested in the Ecotron trial at $n=5$ replicates per fertiliser per climate. In addition, $n=2$ plants per climate were left unfertilised (Table 2). The BBFs were numbered sequentially from one to four for the Ecotron trial. BBF1 (UNI1, UNIVPM) was derived from organic residues of shellfish and fish processing waste in the Adriatic Sea through enzymatic hydrolysis, resulting in a liquid hydrolysate. It contained moderate nitrogen (48.2 g kg^{-1}), low phosphorus (3.25 g kg^{-1}), and moderate potassium (14.4 g kg^{-1}). BBF2 (CAT1, CATAR), was sourced from Atlantic Ocean fish processing waste (heads, bones, viscera), which underwent enzymatic hydrolysis, thermo-mechano-chemical (TMC) fractionation by twin-screw extrusion, and drying to produce a solid protein fraction. Its low water content (2.13%) and high organic matter (84.45%) complement its nutrient-rich profile: high nitrogen (80.71 g kg^{-1}), high phosphorus (50.21 g kg^{-1}), and moderate potassium (11.08 g kg^{-1}). BBF3 (FER3, FERTINAGRO), produced from Cantabrian Sea fish viscera, was processed via membrane separation and autolysis into a slurry NPK solution enriched with amino acids. It had a balanced nutrient profile with high nitrogen (64.07 g kg^{-1}), moderate phosphorus (28.57 g kg^{-1}), and high potassium (46.72 g kg^{-1}). BBF4 (FSP, NIBIO) originated from North Sea dried fish sludge and was processed through drying and palletisation into a solid fish sludge pellet. It contained moderate nitrogen (48.50 g kg^{-1}), moderate phosphorus (32.56 g kg^{-1}), and low potassium (5.96 g kg^{-1}). The synthetic fertiliser (SYN) was a standard liquid nutrient solution formulated by blending and stabilizing nutrients (NO_3 , NH_4 , urea, P_2O_5 , K_2O , and trace elements like Cu, Fe, Mn, Mo, Zn) with EDTA, yielding a classic horticultural fertiliser nutrient composition of 70 g kg^{-1} nitrogen, 30 g kg^{-1} phosphorus, and 50 g kg^{-1} potassium.

Table 2: Characterisation of the four bio-based fertilisers (BBFs) and the synthetic fertiliser (SYN) used in the Ecotron trial with details on respective marine sourcing region, raw input material, technology, final product classification and chemical composition as determined in D6.1.

Fertiliser ID (S2L ID)	Sourcing region	Raw material	Technology	Fertilising product	Water content (%)	Organic matter (%)	Nitrogen (N g kg ⁻¹)	Phosphorous (P g kg ⁻¹)	Potassium (K g kg ⁻¹)
BBF1 (UNI1, UNIVPM)	Adriatic sea	Organic residues from shellfish & fish processing	Enzymatic hydrolysis	Hydrolysates (liquid)	57.88 ± 1.71	82.46 ± 0.21	48.2 ± 1.7	3.25 ± 0.09	14.4 ± 0.26
BBF2 (CAT1, CATAR)	Atlantic ocean	Fish processing waste (head, bone, viscera)	Enzymatic hydrolysis, Thermo-Mechano-Chemical (TMC) fractionation by twin-screw extrusion, drying	Protein fraction (solid)	2.13 ± 0.21	84.45 ± 0.58	80.71 ± 4.3	50.21 ± 6.99	11.08 ± 0.29
BBF3 (FER3, FERTINAGRO)	Cantabrian sea	Fish viscera from fish processing industry	Membrane separation, autolysis	NPK solution w/ amino acids (slurry)	49.42 ± 0.27	83.51 ± 0.24	64.07 ± 0.3	28.57 ± 0.88	46.72 ± 2.03
BBF4 (FSP, NIBIO)	North Sea	Dried fish sludge	Drying, pelletisation	Fish sludge pellet (solid)	17.46 ± 0.42	82.55 ± 0.07	48.50 ± 2.8	32.56 ± 2.26	5.96 ± 0.67
SYN	Synthetic	NO ₃ , NH ₄ , Urea, P ₂ O ₅ , K ₂ O, Cu, Fe, Mn, Mo, Zn	Blending, stabilisation (EDTA)	Nutrient solution (liquid)	79	-	70	30	50

1.4 Experimental design

The TERRA-Ecotron has six controlled environment rooms (CERs), in each of which nine cubes can be placed, resulting in a total of n=54 experimental units bounded by n=9 per room. In this experiment, two climate scenarios were tested, each of which was replicated in three rooms (hence 27 cubes per climate). Further, the experimental design comprised five fertiliser modalities each tested under both climatic conditions. The fertiliser modalities were four BBFs and one synthetic fertiliser, both studied at n=5 replicates per climate, resulting in n=50 experimental cubes. The remaining four cubes were distributed between the two climates and used for negative controls of unfertilised crop (n=2 replicates per climate). The five replicates per fertiliser treatment per climate were equally distributed amongst the controlled environment rooms in a completely randomised block design (Figure 3).

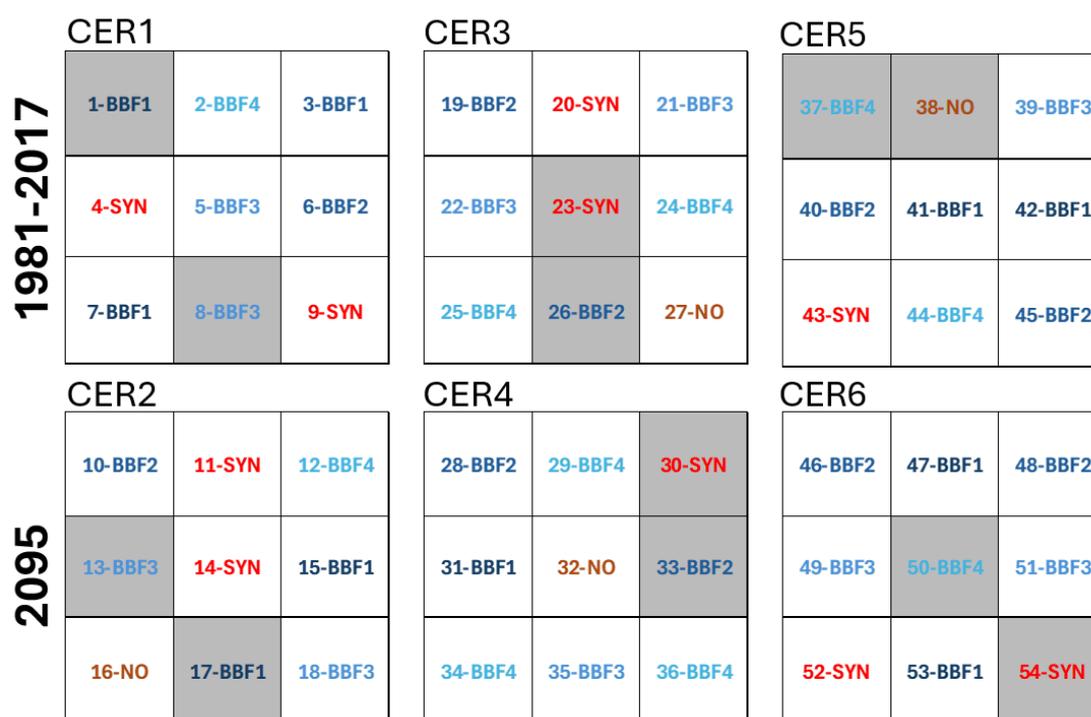


Figure 3: Distribution of the cubes amongst CERs and climates. Grey shading indicates positioning of a scale underneath the cube to improve estimates of evapotranspiration. SYN = synthetic fertiliser, NO = no fertiliser. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

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1.5 Crops investigated

In line with the field experiments, the common crop broccoli was the first crop studied. Broccoli seedlings (*Brassica oleraceae capitata* var. *Koros F1*, floramedia/vivavera, DECA6T1133, BE 2.185.450.877) were planted at the two-leaf stage on 2nd of May 2024 with one plant per cube one day after soil fertilisation with the BBFs and synthetic fertiliser respectively. The broccoli was cultivated over a period of eight weeks within the climate-controlled environments, starting on 21st of April and ending on 23rd of June in simulated time. These simulated dates correspond to a real-life planting date of 2nd of May and a harvest date of 3rd of July 2024. Plants were inspected regularly to monitor crop development and weeds. Cubes were weeded manually when needed and no chemical plant protection products were applied. Plant performance was satisfactory even during the dry spells so that no additional irrigation was applied. After broccoli harvest, spinach (*Spinachia oleracea* var. *Butterflay*, Vilmorin Bio 4655B, Lot JO7227) was sown at a density of 0.56 g seeds per cube (= 2.24 g m⁻²) and harvested after 37 days on 9th of August 2024.



Figure 4: Broccoli (*Brassica oleraceae capitata* var. *Koros F1*, floramedia/vivavera, DECA6T1133, BE 2.185.450.877, left) and spinach (*Spinachia oleracea* var. *Butterflay*, Vilmorin Bio 4655B, Lot JO7227) plants during the Ecotron trial.

1.6 Parameters measured to quantify agronomic performance and environmental impact of the main crop

In addition to the environmental parameters controlled by the Ecotron, several plant and soil parameters were measured regularly during the course of the experiment to quantify agronomic performance and environmental impact of the different fertilisers tested (Table 4).

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Table 4: Crop and soil parameter monitored during the Ecotron trial.

Parameter	Interpretation	Scale of inference / method	Reference
Nitrogen Balance Index (NBI)	Ratio between leaf chlorophyll and flavanol as indicator of plant N-stress	n=3 measurements per plant, n=5 reps per modality, 1x week, via Dualex 4 Scientific (Metos, Pressel Instruments, Weiz, Austria)	Cerovic et al., 2015
Chlorophyll fluorescence (Fv/Fm)	Ratio between variable fluorescence (Fv) and maximum fluorescence (Fm), indication of the maximum efficiency (quantum yield) of photosystem II (PSII), decrease in Fv/Fm can indicate photodamage/ physiological plant stress	n=3 measurements per plant, n=5 reps per modality, 1x week, via Handy PEA + (Hansatech Instruments Ltd, Norfolk, United Kingdom)	Vlaović et al., 2020
Canopy area (cm²)	Surface cover of plant biomass, links to transpiration & photosynthesis potential	1x week, n=5 reps per modality, via ImageJ	Schneider et al., 2012
BBCH growth stages	Standardised scale used to describe the phenological development of plants	1x week, n=5 reps per modality, visual assessment	Meier, 2001
Plant biomass (g) & head diameter (cm)	Plant growth and biomass allocation between plant compartments (heads, stems, leaves, roots), marketable yield	1x at harvest, n=5 reps per modality	n.a.
Soil N₂O and CO₂ emissions (μmol m⁻² s⁻¹)	GHG emissions from the cropping systems, related to soil microbial processes metabolising plant, soil and fertiliser molecules	1x week, n=5 reps per modality, via Licor LI-850 & Licor LI-7820 (LI-COR Inc., Lincoln, USA) with permanently installed respiration chambers (h=7.5cm, d=10cm)	Cowan et al., 2019
Soil enzymatic activity (nmol g⁻¹ h⁻¹)	Hydrolysis of fluorescein diacetate (FDA) as proxy of soil microbial activity	Quantified on composite soil samples of every modality (BBF x climate) at	Green et al., 2006; Adam et al., 2001

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		1, 2, 3, 6 and 8 weeks after fertilisation	
Aqueous nitrate in interstitial soil pore water (mg L⁻¹)	Availability of water-soluble nitrate in the cropping system, linked to plant N-uptake, microbial processes, can indicate risk of N-leaching	1x week unless too dry, water extracted from each cube via permanently installed rhizons (Rhizosphere Research Products B.V., Wageningen, The Netherlands), NO ₃ quantification via LAQUAtwin NO3-11C (Horiba Ltd., Kyoto, Japan)	Folegatti et al., 2005
Soil temperature (°C) & humidity (%VMC)	Function of environmental parameter and plant water uptake	3 sensors (Ecotechnique SM150T, Delta-T devices, Cambridge, UK) per climate, 1 sensor per CER, in one cube, depth = 15cm, records every 5 mins	n.a.
Soil matric potential (hPa)	Function of environmental parameter and plant water uptake	2 sensors (SMS-SKT 850, SDEC, Reignac-sur-Indre, France) per climate (CERs 2,3,5,6), in one cube, depth = 15cm, records every 5 mins	n.a.

2 Impact of fertiliser & climate on cropping system

2.1 Plant biomass production and marketable yield

Overall, crop performance was statistically not different ($p > 0.05$) between BBFs and the synthetic fertiliser (SYN), especially marketable yield (fresh weight of broccoli heads and head diameter) with BBF2 (CAT1) and BBF3 (FER3) in the historic climate, and with BBF1 (UNI1), BBF3 (FER3) and BBF4 (FSP) in the future climate reached levels comparable to plants fertilised with SYN. Overall, the future climate decreased plant growth and impacted all cropping systems negatively irrespective of the fertiliser used, with BBF2 (CAT1) suffering the most from climate induced yield penalties (Figure 4, Figure 5, Table 5).

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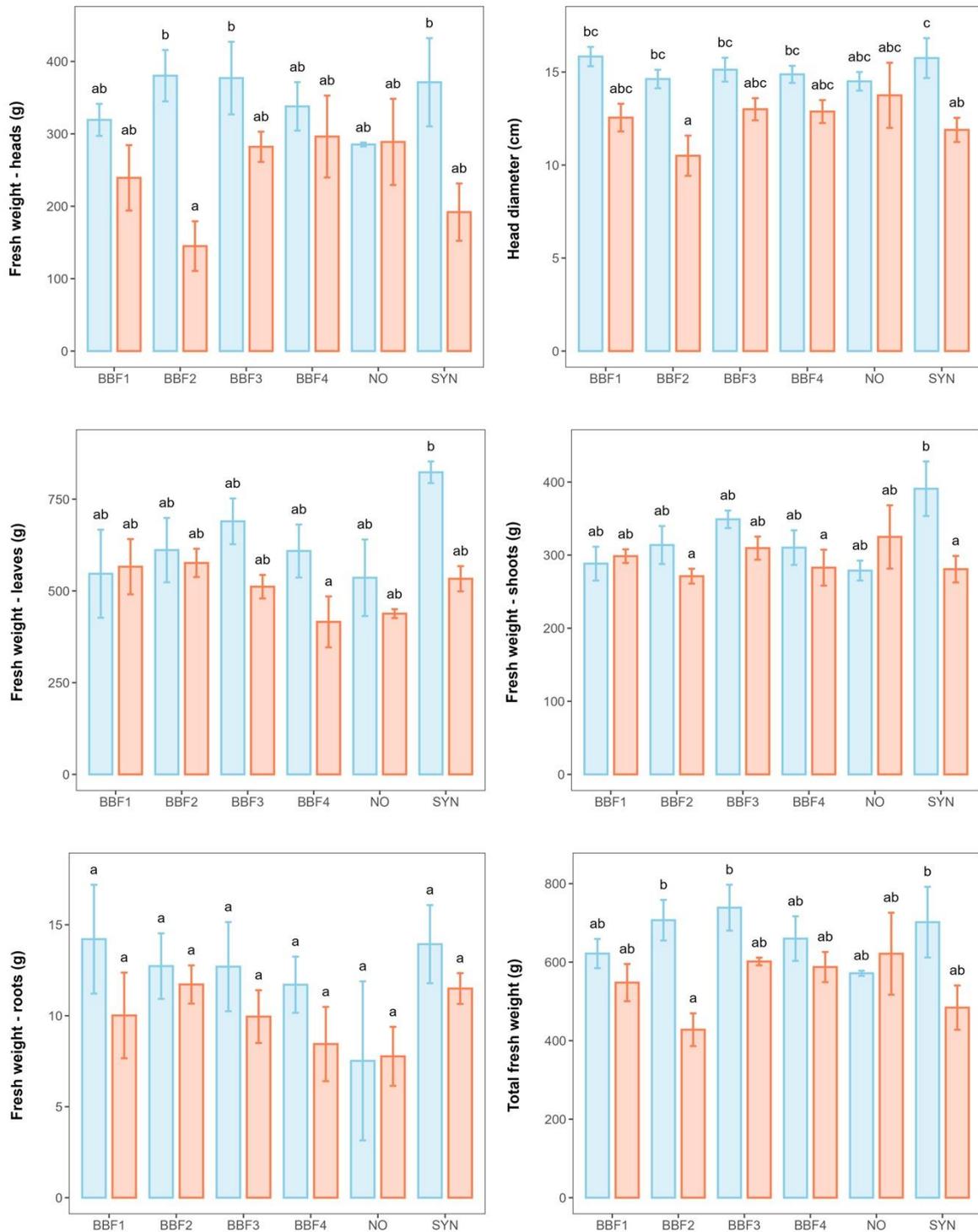


Figure 4: Fresh weights of broccoli plant compartments and head diameter at harvest.

Blue under historic reference climate (1981-2017) and orange under future climate (2095). Letters indicate grouping across fertiliser and climate treatments based on post-hoc Tukey's test and analysis of variance. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

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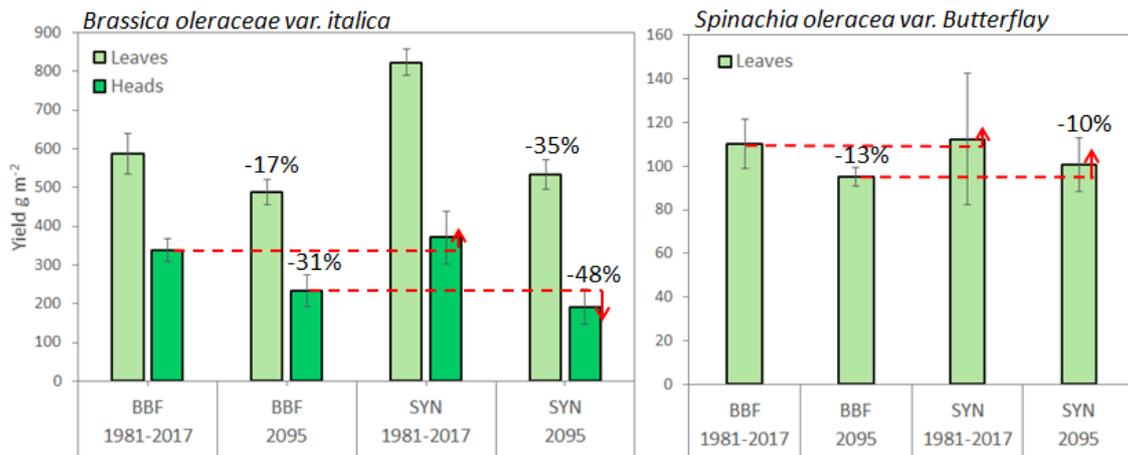


Figure 5: Yield (g fresh weight m⁻²) of broccoli directly fertilised with BBFs & SYN, and spinach sown after broccoli harvest without additional fertiliser. Red lines indicate comparison between average BBF yield (all four BBFs combined) and SYN for each climate. Numerical values provided in figure are relative decrease in yield caused by climate for the same fertiliser type.

Table 5: Results of analysis of variance for the two main factors fertiliser treatment and climate, and their interaction for the different plant biomass parameter of broccoli and spinach.

Broccoli					
	Head fresh weight (g)		Head diameter (cm)		
	F	p	F	p	
Fertiliser	0.983	0.44	1.142	0.358	
Climate	16.257	< 0.001	30.217	< 0.001	
Ferti x clima	2.233	0.07	1.026	0.418	
	Leaves fresh weight (g)		Shoot fresh weight (g)		
	F	p	F	p	
Fertiliser	1.718	0.152	1.834	0.128	
Climate	11.194	0.002	4.447	0.041	
Ferti x clima	1.037	0.408	1.966	0.104	
	Roots fresh weight (g)		Total fresh weight (g)		
	F	p	F	p	
Fertiliser	1.063	0.395	1.623	0.174	
Climate	3.584	0.065	10.468	0.002	
Ferti x clima	0.183	0.968	2.5	0.045	
Spinach					
	Leaves fresh weight (g)		Roots fresh weight (g)		
	F	p	F	p	
Fertiliser	1.033	0.411	1.857	0.123	
Climate	1.216	0.277	0.084	0.774	
Ferti x clima	0.136	0.983	0.202	0.96	

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2.2 Cropping system behaviour and environmental impact of BBFs in broccoli production under two climate scenarios

Several parameters were measured to evaluate the performance of the main crop broccoli under the different fertilisers in both climate scenarios (Figure 6). At leaf level, nitrogen balance index (NBI) and chlorophyll fluorescence (Fv/Fm) were regularly measured to determine plant stress. NBI captures the ratio between chlorophyll and flavanol content in plant leaves, where a decrease is generally interpreted as impoverishment of plant nitrogen nutrition and/or other stress (Cerovic et al., 2015).

Chlorophyll fluorescence was also measured weekly to estimate plant stress based on photosynthetic efficiency. Here, the stress response of plants is measured through a pulse-amplitude modulation (pulse of saturating light after dark adaptation) and calculated as the ratio between variable fluorescence (Fv) and maximum fluorescence (Fm), an indication of the maximum efficiency (quantum yield) of photosystem II (PSII). The ratio between Fv and Fm varies between 0 and 1, with a healthy range between 0.75 and 0.85. A decrease indicates plant stress and/or photodamage affecting the PSII reaction centre (Vlaović et al., 2020).

Moreover, BBCH growth stages and canopy cover were regularly recorded as non-destructive measures to track aboveground crop biomass production (Figure 6). From day 143 onwards, plants in the future climate were in phenological advance compared to plants in the reference climate (higher BBCH on similar dates, $p < 0.05$), but no significant difference was observed between fertiliser treatments. Canopy area was significantly different between fertiliser treatments ($p = 0.007$), and between climates ($p < 0.001$). Considerable temporal variability in NBI and Fv/Fm resulted in overall no significant differences between fertiliser treatments or climates, however occasionally significant ($p < 0.05$) drops were observed for both parameters (e.g. at d134 for NBI or at d154 for Fv/Fm).

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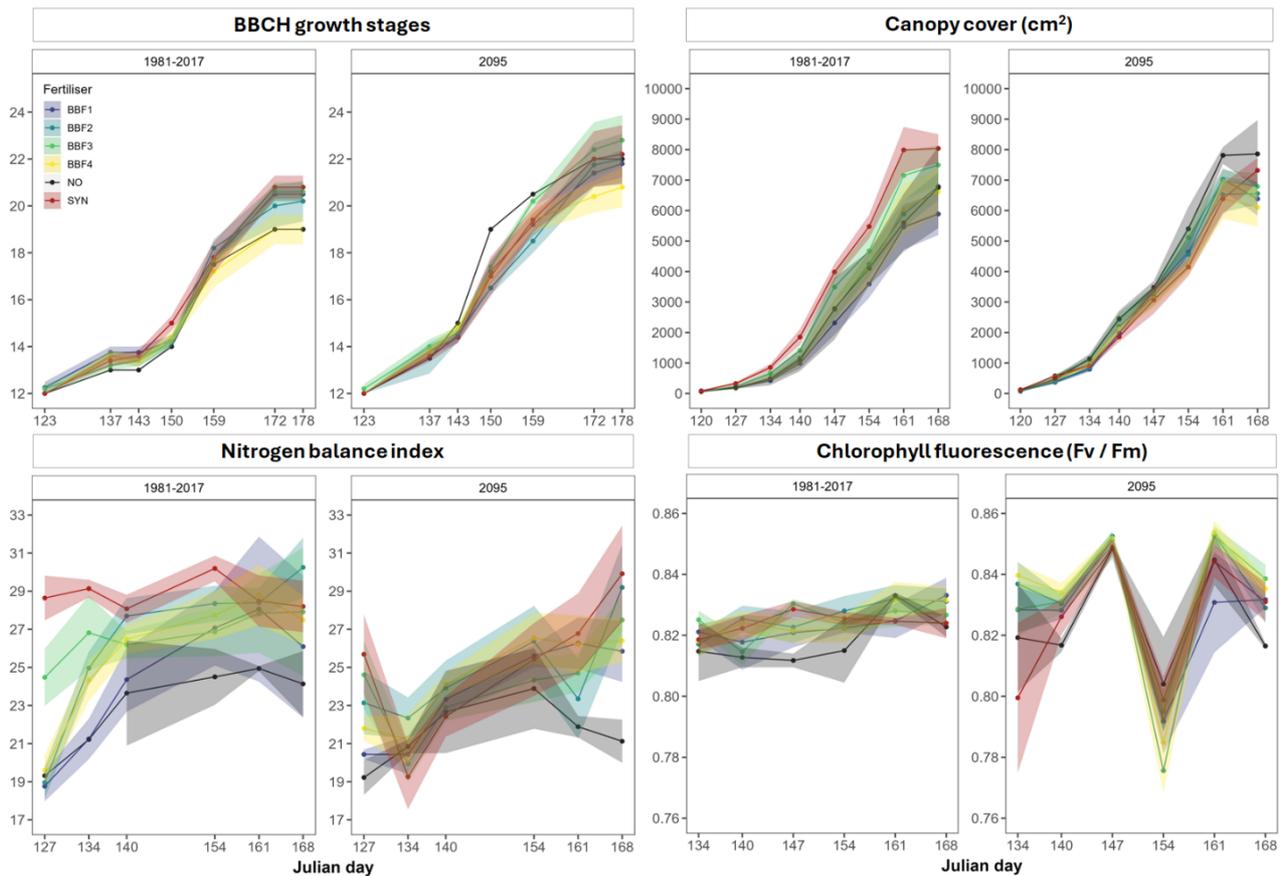


Figure 6: BBCH growth stages, nitrogen balance index (NBI), canopy area (cm²) and chlorophyll fluorescence (Fv/Fm) of broccoli plants during the crop growth season in the two climate scenarios. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

In addition, soil microbial activity and soil nitrate concentration were measured over time to track the impact of BBFs on belowground processes (Figure 7). Microbial activity was approximated by measuring hydrolysis of fluorescein diacetate (FDA) on composite soil samples and nitrate was measured in extractable interstitial soil pore water extracted from each cube weekly, if conditions allowed i.e. when the soil is not too dry. Soil microbial activity was lower in the future climate than in the historic reference climate ($p < 0.001$) and significantly different between fertiliser treatments, with significantly higher enzymatic activity with BBF1 (UNI1), and significantly lower activity with SYN and NO fertiliser ($p = 0.03$). Soil nitrate could only be quantified when interstitial soil pore water was extractable, and hence not possible at the later timepoints in the future climate due to dry soil conditions caused by more intense alterations of dry-rain spells at higher temperatures (Figure 1, Figure 8). The average water extractability was 61.11% in the historic reference climate and 15.74% in the future climate scenario.

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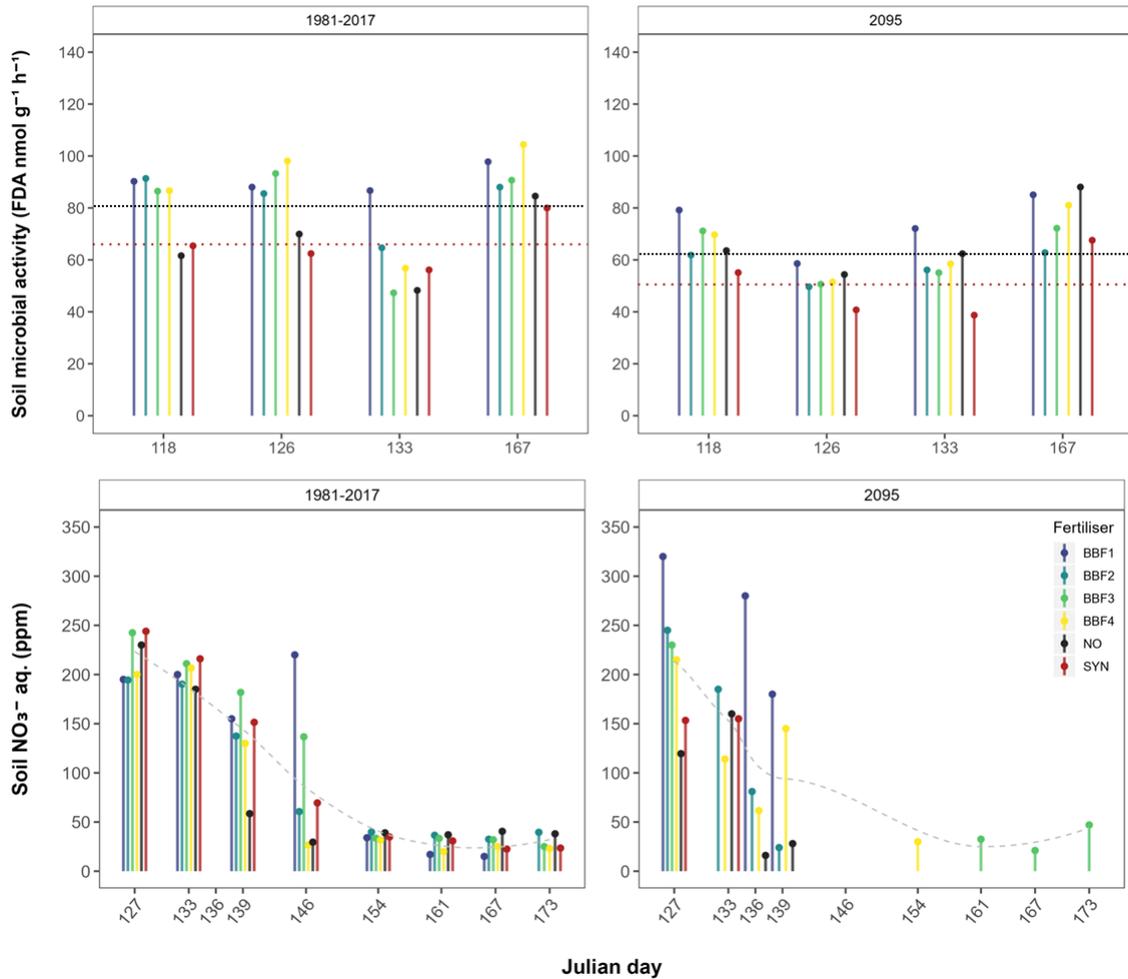


Figure 7: Average soil microbial activity and soil nitrate concentration measured over time by fertiliser type and climate. Black dotted lines are means across all BBFs and red dotted lines are means of SYN. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

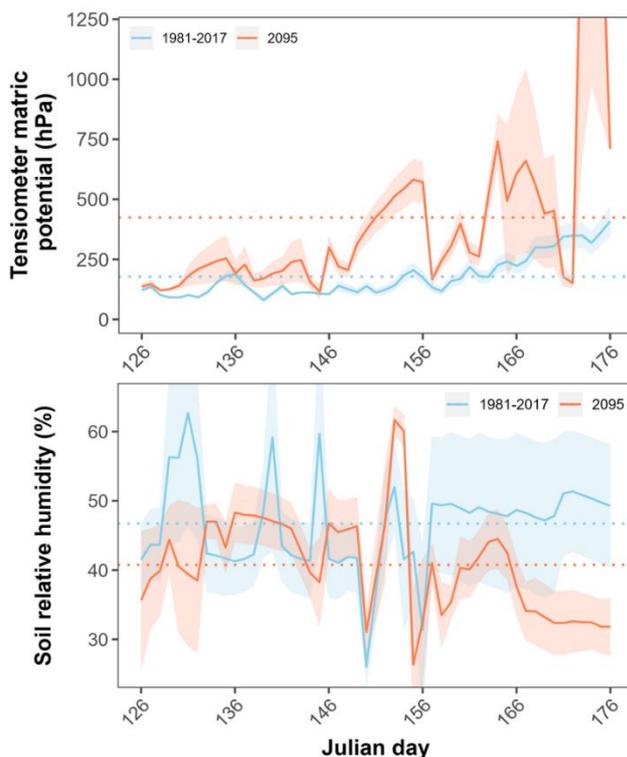


Figure 8: Soil water dynamics. Top panel shows soil matric potential (hPa) and lower panel shows relative soil humidity (%). Solid lines are daily means (\pm SD) or, when only one sensor per climate recorded data, a 30% standard deviation was plotted to indicate the error range of the measurement. Dotted lines represent means across all data points for each climate. For matric potential, $n=2$ sensors (Tensiometer SMS-SKT 850, SDEC, Reignac-sur-Indre, France) per climate were installed (CERs2,3,5,6). For humidity, solid lines are daily means (\pm SD) for $n=3$ sensors (Ecotechnique SM150T, Delta-T devices, Cambridge, UK) per climate (one per CER).

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Finally, nitrogen and phosphorous use efficiencies (NUE, PUE) were determined at the end of the main crop growth season as the ratio between respective element contents in plant biomass and the amount of element available from soil and fertiliser (Figure 9). NUE and PUE were overall not significantly different between fertiliser treatments, and while NUE showed variable shifts in response to climate depending on the fertiliser used, PUE on average decreased in the future climate for all BBFs with no change in PUE in absence of fertiliser.

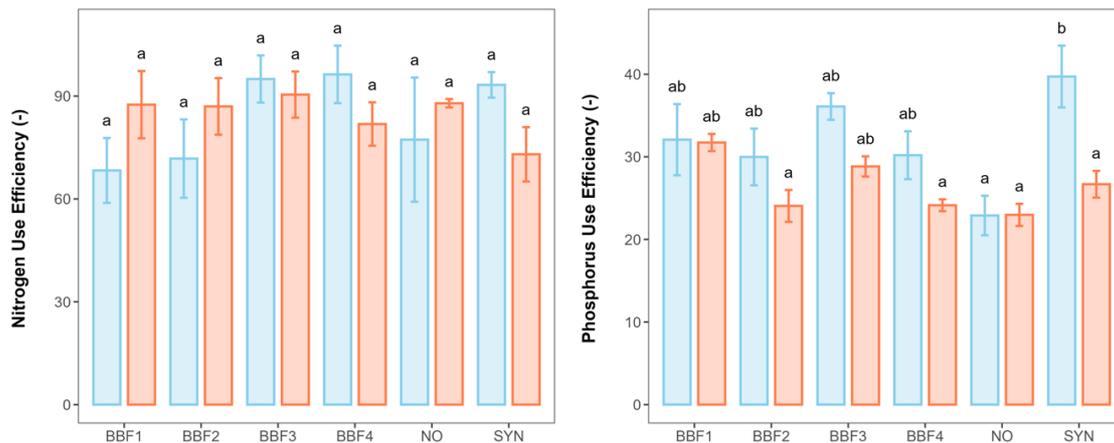


Figure 9: Nitrogen use efficiency (NUE) and phosphorous use efficiency (PUE) of broccoli plants fertilised with either of the four different BBFs, the synthetic fertiliser (SYN) or without fertilisation (NO). Blue under historic reference climate (1981-2017) and orange under future climate (2095). Letters indicate grouping across fertiliser and climate treatments based on post-hoc Tukey's test and analysis of variance. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

2.3 Detailed comparison of BBFs with synthetic fertiliser

To further evaluate the market potential of BBFs, crop performance of plants receiving BBFs was compared to those receiving synthetic fertiliser (SYN) for both climate scenarios (Figure 10). Parameters included (A) Nitrogen Use Efficiency (NUE), (B) Phosphorous Use Efficiency (PUE), (C) Head diameter (cm), (D) Head fresh weight (g), (E) Total plant biomass (g dwt), (F) Cumulative nitrous oxide (N₂O) and (G) carbon dioxide (CO₂) fluxes (μmol m⁻²). Panels show effect sizes of pair-wise comparisons between each of the BBFs with the synthetic fertiliser (SYN), the value provided corresponds to the mean effect of BBF compared to SYN on respective parameter under respective climate. If the comparison is in the green shaded area, the BBF performs better than SYN. For example, broccoli fertilised with BBF1 (UNI1) gives approximately +0.31cm larger heads than broccoli fertilised with SYN in the historic reference climate, and +0.59cm in the future climate. For N₂O and CO₂, if the values fall in the red-shaded area, the emissions are

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higher for BBFs compared to SYN. For each pair-wise comparison, the central dot represents the difference in mean estimate, the thin and thick line represents the 95% and 75% credible intervals.

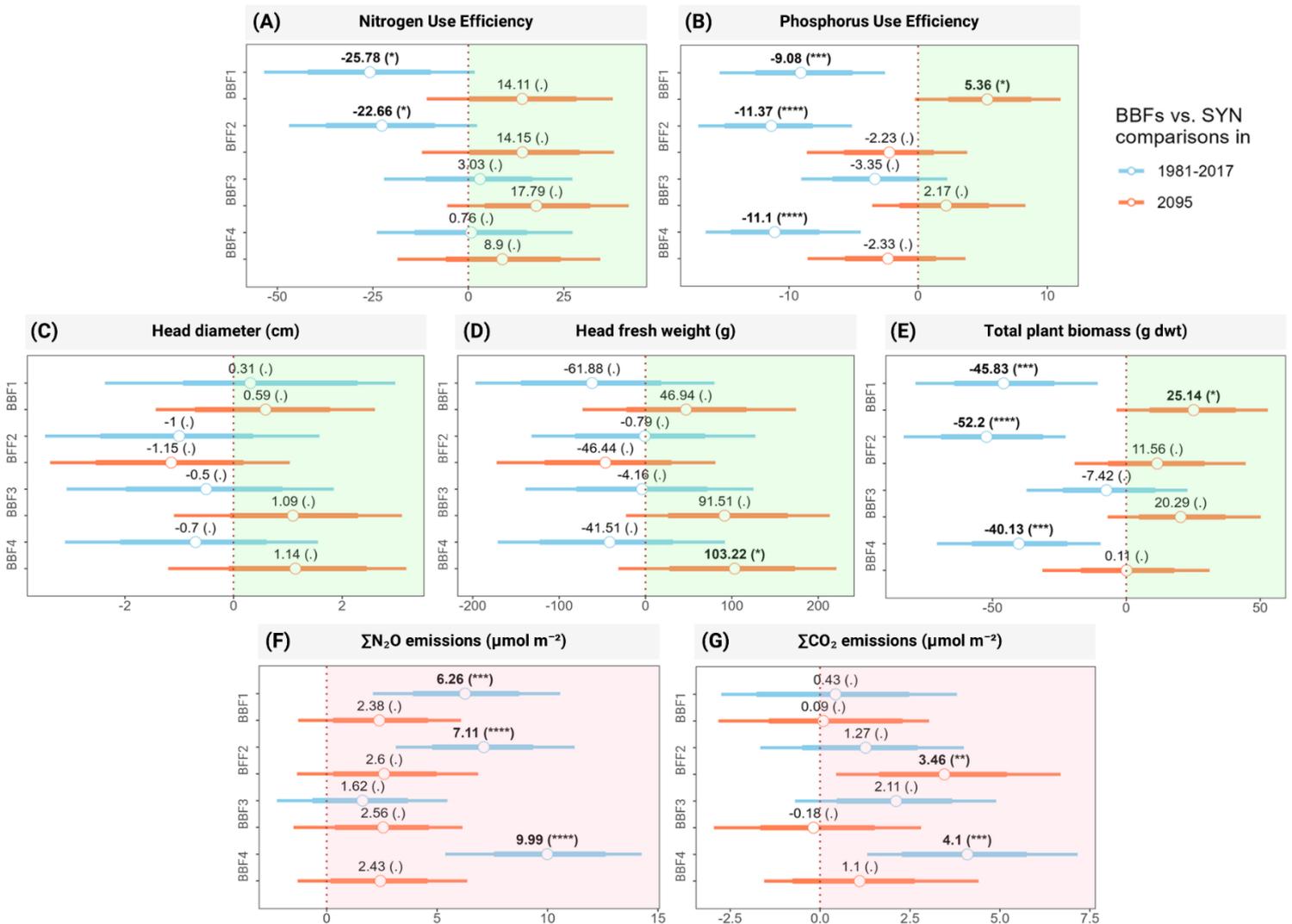


Figure 10: Pairwise comparisons between each of four bio-based fertilisers and the synthetic fertiliser (BBF1-4 x SYN) in the two climate scenarios. (A) Nitrogen Use Efficiency (NUE), (B) Phosphorous Use Efficiency (PUE), (C) Head diameter (cm), (D) Head fresh weight (g), (E) Total plant biomass (g dwt), (F) Cumulative nitrous oxide (N_2O) and (G) carbon dioxide (CO_2) fluxes ($\mu\text{mol m}^{-2}$) under broccoli cultivation in historic climate (blue) and predicted future climate (orange). Panels show effect sizes of pair-wise comparisons for each BBF with the synthetic control fertiliser (SYN). The value provided corresponds to the mean effect of BBF compared to SYN for each parameter under both climates. For each pair-wise comparison, the probability of direction (pd) is indicated with asterisk according to effect existence levels (****) certain (pd > 0.999), (***) probable (pd > 0.99), (**) likely (pd > 0.97), (*) possible (pd > 0.95), (.) uncertain (pd ≤ 0.95). BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

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The four different BBFs tested in the Ecotron trial showed variable sensitivity to the future climate with both improvements and deteriorations amongst the agronomic parameters. Meanwhile, the synthetic fertiliser was consistently negatively impacted by the future climate for agronomic parameter with no clear trend for GHG emissions. Thus, the gap in agronomic performance between SYN and BBFs was closed or even reversed in the future climate scenario. Notably, BBF1 (UNI1: liquid shellfish/fish hydrolysates) and BBF3 (FER3: NPK slurry solution with amino acids from fish viscera) displayed enhanced performance in key parameters such as total biomass and broccoli head fresh weight when compared to SYN under the future climate. The biggest increase in head fresh weight in the future climate was achieved with BBF4 (FSP: fish sludge pellet), which otherwise had no significant difference to SYN in agronomic performance or GHGs and is by these means a promising substitute. Conversely, in this experiment BBF2 (CAT1: solid protein fraction) tended to be overall the least effective fertiliser compared to SYN with the highest number of reduced plant performance parameter and the lowest microbial activity of all here tested BBFs. Yet, overall BBFs positively impacted crop growth in this study, especially under future climate conditions. This is in line with previous experiments evaluating the use of BBFs with horticultural and cereal crops. For example, it has been shown that combining BBFs with microbial inoculation can significantly increase yields of tomato plants compared to synthetic fertiliser (Clagnan et al., 2023) and in a two-year crop cycle of winter wheat and ryegrass, commercially available BBFs reached 47-80% replacement value for nitrogen and 105-161% for phosphorous (Müller et al., 2024). This is in line with the results of this study, where fertiliser addition rates were normalised to 120kg N ha⁻¹ for all treatments, which resulted in lower NUE in plants fertilised with BBF1 (UNI1) and BBF2 (CAT1) compared to SYN in the historic reference climate. However, NUE in the future climate was systematically increased for all BBFs compared to SYN, with a similar trend observed for phosphorous. This demonstrates the improved market potential of BBFs under future climates and highlights their potential role in sustainable and circular agricultural production in the future.

Moreover, the Ecotron trial confirmed consistent declines in yield under future climatic conditions and highlights worsened market-relevant parameters such as broccoli fresh weight and head diameter across all fertiliser treatments, including synthetic fertiliser. These observations are in line with a previous study which demonstrated that the climatic conditions expected under the RCP8.5 scenario would exceed the capacity of broccoli plants to adapt, highlighting in particular growth inhibition and reduction in plant vigour with a major impact on yield (Pineda et al., 2024). Similar observations have been made for broccoli in the field. For example, during the strong summer drought after the wet

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spring in 2018, the Irish horticultural sector lost 15% of its broccoli production to poor plant development and crop failures where no irrigation was available (Grove, 2019). Fertiliser optimisation, together with improved irrigation systems, can play a critical role in mitigating such yield losses. Especially BBFs in pelleted and slurry formulations can release nutrients slowly and thus be more effective during seasons with more intense rain and drought events (Müller et al., 2024; Lawrencía et al., 2021). However, the efficiency of BBFs also depends on the capacity of the soil microbial communities to degrade the biomolecules and release their mineral elements (Clagnan et al., 2023; Chaturvedi et al., 2012). As demonstrated in this experiment, in the right pedo-climatic context, BBFs can enhance soil microbial activity and physical characteristics and thus help maintain crop productivity even under stress conditions (Chaturvedi et al., 2012; Wester-Larsen et al., 2024). Further fine-tuning the interactions between BBFs, crop species and microbial communities, combined with advanced irrigation technologies, are hence promising pathways to address climate-induced yield losses while increasing nutrient retention in the cropping system and improving the environmental footprint (Clagnan et al., 2023; Cui et al., 2018).

2.4 Nutrient exports in harvested products per area

As a further measure to evaluate the environmental impact, nutrient exports in aboveground biomass per cropped area were calculated for all fertiliser x climate modalities (Figure 11). On average, nutrient exports per area were mostly indifferent between the different fertiliser treatments and showed variable shifts between the climates within each fertiliser treatment, with no consistent overall trend. This underscores the complexity of nutrient dynamics in crop production under changing environmental conditions and the need for adaptive agricultural practices to optimize nutrient management and minimize environmental impacts while ensuring sufficient and sustainable crop production.

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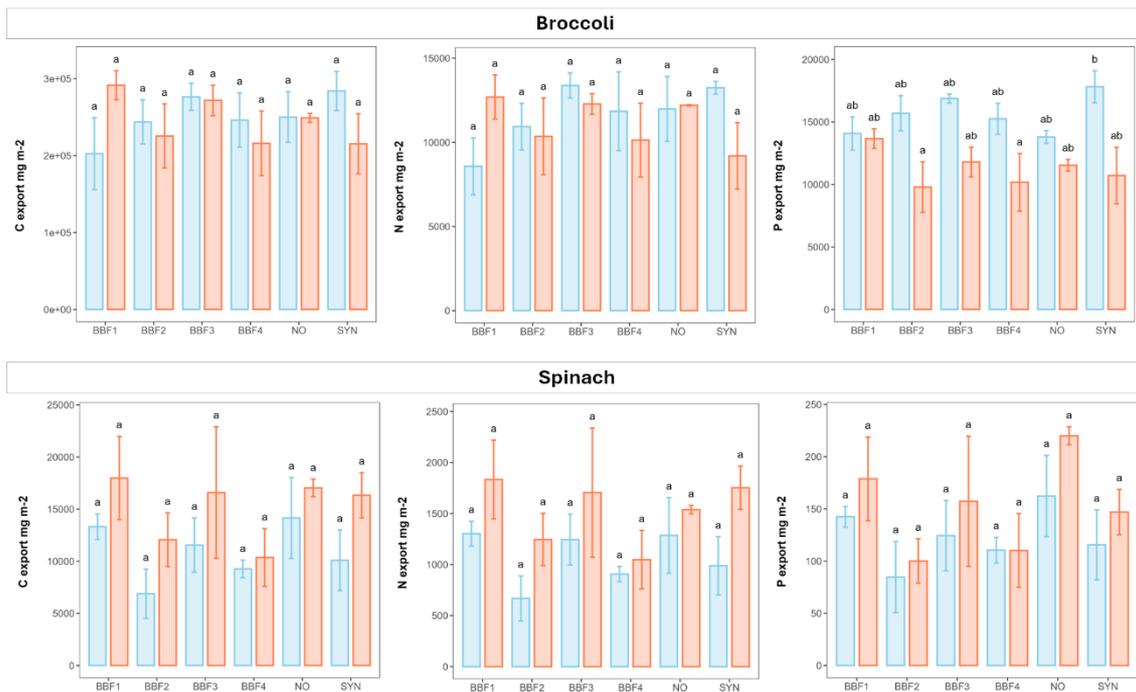


Figure 11: Nutrient exports (C, N, P) in harvested products per area. Blue under historic reference climate (1981-2017) and orange under future climate (2095). Letters indicate grouping across fertiliser and climate treatments based on post-hoc Tukey's test and analysis of variance. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

2.5 Nutrient composition (C:N:P) in harvested products

Finally, the nutrient composition (C, N, P) of the marketable products was determined. There were no significant differences between fertiliser treatments or climates, but for both crops the C:N ratio tended to decrease in the future climate, while C:P and N:P ratios increased under future climatic conditions (Figure 12). The higher nitrogen content relative to carbon in the future climate can result in food products with higher protein content as nitrogen is a key component of amino acids, the building blocks of proteins. For human health, this can be beneficial, however, excessively high nitrogen levels in plants can lead to an accumulation of nitrates, particularly in leafy vegetables like spinach. High dietary nitrate intake may pose health risks and should therefore be monitored. The increased C:P ratio suggests a lower phosphorus content relative to carbon in plants, which could potentially lead to phosphorous deficiencies, particularly in populations reliant on plant-based diets.

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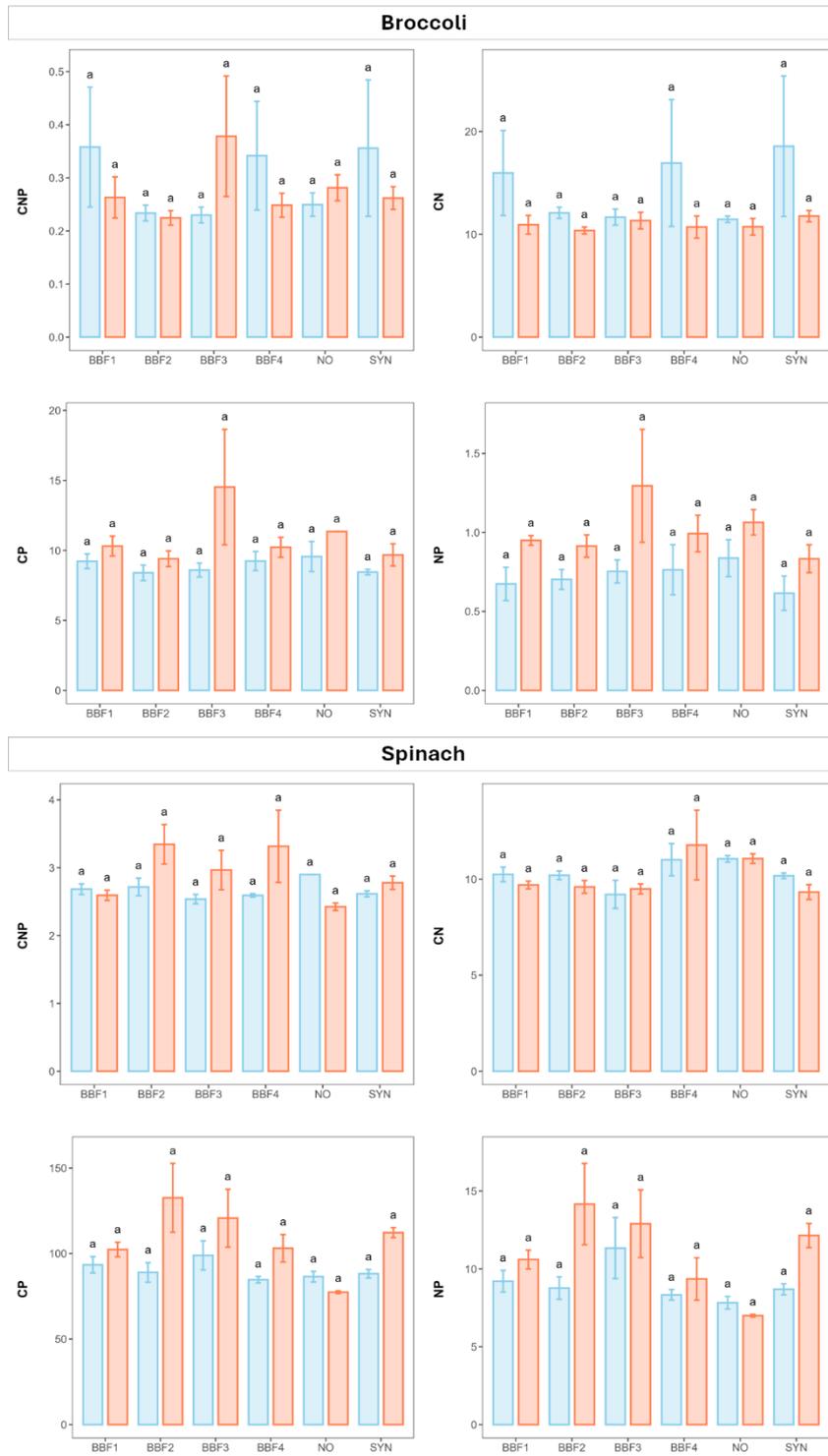


Figure 12: Elemental composition (C:N:P) of broccoli heads (top four panels) and spinach (bottom four panels). Blue under historic reference climate (1981-2017) and orange under future climate (2095). Letters indicate grouping across fertiliser and climate treatments based on post-hoc Tukey's test and analysis of variance. BBFs: BBF1 (UNI1, UNIVPM), BBF2 (CAT1, CATAR), BBF3 (FER3, FERTINAGRO), BBF4 (FSP, NIBIO).

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3 Conclusion

The Ecotron trial demonstrated the feasibility of BBFs as agronomically performant alternatives to SYN, with most BBFs achieving yields comparable to yields under synthetic fertiliser, particularly under the future climate scenario. To further define the conditions under which the application of BBFs can be most beneficial for crop growth, future studies could investigate the efficiency of BBFs over a broader gradient of pedo-climatic contexts and also take the aggregate state of the different fertilisers into account, i.e. whether the formulation is solid, liquid or a slurry, as this may affect nutrient release dynamics. To reduce the environmental impact, future studies could also address greenhouse gas emissions of systems under BBFs. Overall, addressing climate-induced yield penalties observed for all fertilisers will be critical to ensure food security for a growing world population in the face of climate change.

4 Data provided for task 5.3 Simulation modelling

All environmental and agronomic data has been delivered to task 5.3 to test the predictions of the DNDC model, which has been parametrized based on the data from field trials (task 5.2).

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