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Assessing the environmental behavior of alternative fertigation methods in soilless systems: The case of *Phaseolus vulgaris* with Struvite and Rhizobia inoculation.

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Highlights:

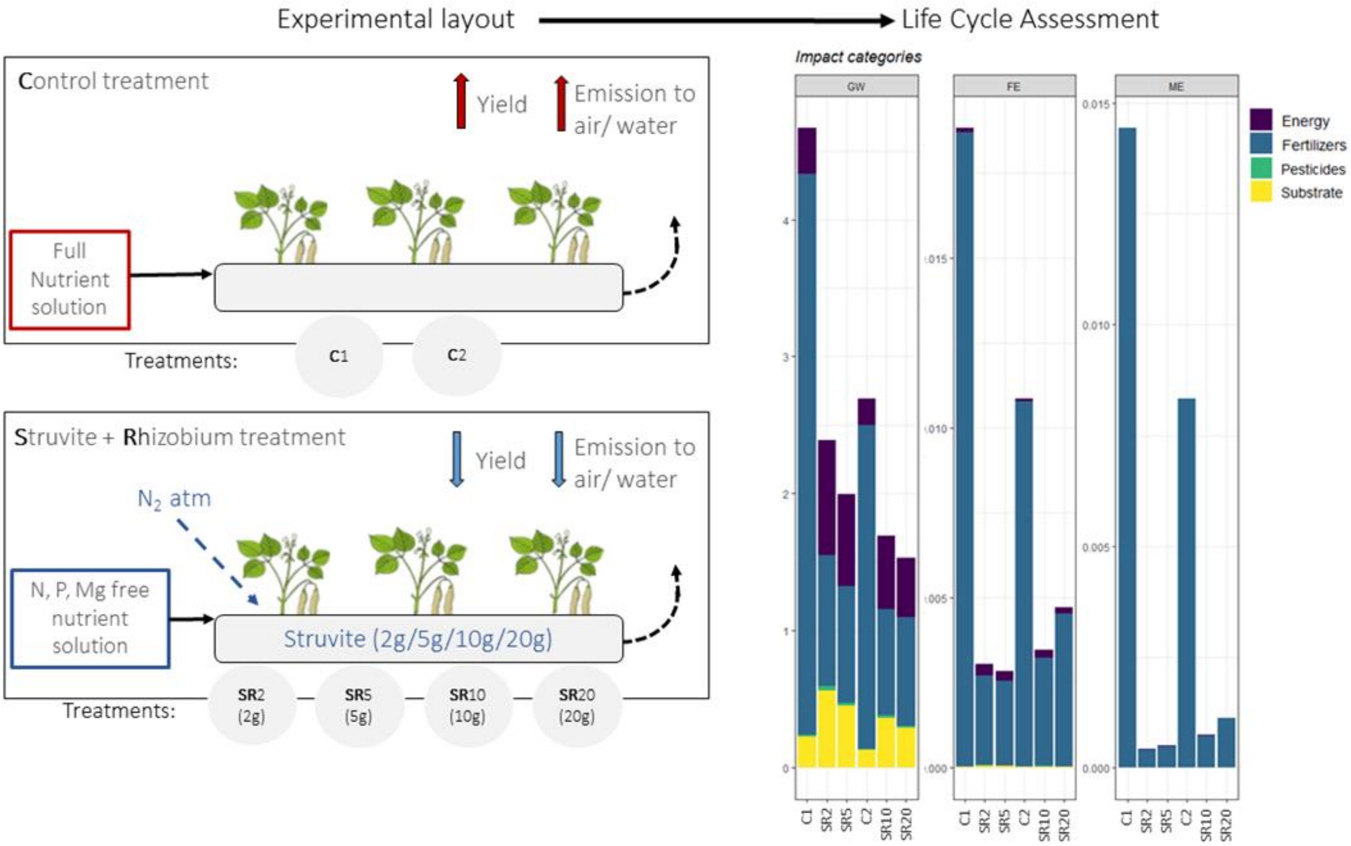
- Inorganic fertilization can be a great constrain in the implementation of Urban Agriculture due to increasing emissions to the environment.
- Alternative fertilization methods using struvite and nitrogen fixing bacteria can be considered to avoid additional impacts in common bean production.
- Albeit alternative fertilization result in lower yields than conventional methods, life cycle impacts are significantly reduced.
- Using struvite and nitrogen fixing bacteria reatly reduces quantities of N and P emitted to water.
- Depending on complexity of the infrastructure the yield reduction can be assumed and remain below the control treatment.

Abstract:

Urban agriculture, while being a promising solution to increase food sovereignty in cities, can lead to an unprecedented discharge of nutrient and fertilizer-related emissions into the urban environment. Especially relevant are nitrogen (N) and phosphorus (P), due to their contribution to marine and freshwater eutrophication. Therefore, alternative methods of fertilization need to be put into practice to avoid such impacts to the surrounding environment. Struvite, a precipitate formed in wastewater treatment plants, has been studied as a potential slow releasing fertilizer due to its high content of P, while the bacteria rhizobium has been used to fix N directly from the atmosphere. Legumes, like the common bean are N-demanding crops capable of symbiosis with the bacteria rhizobium and have previously shown positive responses to fertilization with struvite. This study aims to analyze the performance of plant production in a hydroponic system with the combination of rhizobium inoculation and struvite (granulated in four quantities: 2g, 5g, 10g, 20g) irrigated with a N and P deficient nutrient solution, as well as a life cycle analysis (LCA) to determine the possible impact. The nutrient content of in- and out-going irrigation was analyzed as well as in plants and beans. The functional unit for the LCA was 1kg of fresh green beans. The results obtained indicate a yield reduction of 60% to 50% in comparison to the control which was irrigated with a full nutrient solution. The impacts from operational stage are less in all impact categories, where most significant reductions are seen in eutrophication due to lower emissions of N and P into water and air in the operation system. Although the Infrastructure does not

change between treatments, its impacts increase due to lower yields in the alternatively fertilized Bean plants. An important finding is that infrastructure impacts can underscore the benefits of substituting mineral fertilizers. Thus pointing to simple infrastructure to truly reduce environmental impacts for urban agriculture systems.

Graphical Abstract:



Introduction:

Urban Agriculture (UA) has the potential to replace traditional food supply chains to some degree, thereby reducing transportation, packaging and food losses while increasing food sovereignty of cities (Sanyé *et al.*, 2012; Tornaghi, 2017; Sanjuan-Delmás *et al.*, 2018; Siegner, Acey and Sowerwine, 2020). However, the additional need of inorganic chemical fertilizers inevitably results in greater discharge of these chemicals into the environment as well as an increase of the resource depletion potential (Rufí-Salís, Calvo, *et al.*, 2020). This is especially relevant considering the emission of nitrogen and phosphorus species, substantially contributing to marine and freshwater eutrophication, causing oxygen deprivation in aquatic environments. Specifically, urban water cycles and runoff are a great concern with their high implication in water eutrophication damaging ecosystems close to cities as well as close to intensely fertilized agricultural sites (John H. Ryther and Dunstan, 1971; Lewis, Wurtsbaugh and Paerl, 2011). The integration of agriculture within city boundaries could therefore further increase the potential of emissions into the urban water cycles.

It is important to find ways for UA to be highly resource-efficient so that urban areas are able to expand these production practices without incurring significant environmental impacts associated with the additional water, energy, and nutrient requirements. To mitigate these environmental impacts, alternative and more environmentally friendly fertilizers have to be applied to attain competitive yields without causing great impacts to the surrounding environment (Lewis, Wurtsbaugh and Paerl, 2011) as well as avoiding further extraction of phosphorous for agricultural purposes (Linderholm, Tillman and Mattsson, 2012).

Recent work has been focused on the recovery of nutrients from wastewater treatment plants (Harder *et al.*, 2019; Lam, Zlatanović and van der Hoek, 2020; Shaddel *et al.*, 2020), showing a great range of possible alternatives for fertilization generated in urban areas as well as their constraints. One of the available options showing great potential for its use in agriculture, is struvite. The struvite crystal is formed by a spontaneous precipitation in wastewater treatment plants and is regarded as a slow releasing fertilizer due to its low dissolution and high content of phosphorous (12.5%), magnesium (9.9%) and nitrogen (5.7%) (Rahman *et al.*, 2014; Talboys *et al.*, 2016; Degryse, Baird, Rodrigo C da Silva, *et al.*, 2017). %). It has been reported that the formation of struvite can recover up to 90% of Phosphate in wastewater sludge, reaching even higher percentages depending on the precipitation process and source (Kataki *et al.*, 2016).

Studies on the use of struvite in agriculture (Massey, M.S., Davis, J.G., Sheffield, R.E., Ippolito, 2007; Ackerman *et al.*, 2013; Talboys *et al.*, 2016; Degryse, Baird, Rodrigo C. da Silva, *et al.*, 2017) point out that the use of these recovered nutrients can reduce mineral fertilizer requirements while implying little to no cost for farmers (Karak and Bhattacharyya, 2011). The use of struvite has been shown to successfully substitute the use of mineral phosphorous fertilizers, while reducing nutrient losses to the environment due to its slow dissolution rate (Ahmed *et al.*, 2018). Agricultural production with struvite as the main source of P has been tested on a variety of crops rainging from ryegrass (*Lolium perenne*) and broad beans (*Vicia faba*), which experience an increase of fresh yiels of 76% and 54% respectively, to canola (*Brassica napus*) and wheat (*Triticum aestivum L*.), that suffer a reduction of the nutrient uptake and therefore a reduction of the plant yield (Ahmed *et al.*, 2018). Other crops, however, have been seen to experience no significant changes with the use of struvite like the case of maize (Uysal *et al.*, 2014) and corn (Thompson, 2013).

The environmental performance of the struvite extraction as P fertilizer has been previously studied (Ishii and Boyer, 2015) and while its benefits in comparison to virgin phosphorous have been identified in the reduction of nutrient emissions and offsets commercial fertilizer production, its total environmental performance depends on the chemical inputs used for the struvite precipitation as well as the infrastructure and the recovery accounted in the life cycle inventory (Linderholm, Tillman and Mattsson, 2012; Ishii and Boyer, 2015; Lam, Zlatanović and van der Hoek, 2020).

Whereas these studies have mainly substituted phosphorous based fertilizers, the use of nitrogen in the form of ammonium nitrate, urea and monoamonium is still given to the crops. As previously established, the emissions of nitrogen in the environment are greatly damaging and especially crucial for high N demanding crops like legumes.

The inoculation of legume crops with the bacteria rhizobium has been explored as a way for the plant to fix its nitrogen directly from the air without boosting its environmental footprint (Olivera *et al.*, 2004; Gopalakrishnan *et al.*, 2015; Kontopoulou *et al.*, 2015, 2017; Savvas *et al.*, 2018; Araujo, Urbano and González-Andrés, 2020; Sammauria *et al.*, 2020; Sanyal, Osorno and Chatterjee, 2020). This bacteria forms an endosymbiotic interaction with the plant, profiting from compounds generated through photosynthesis while fixing atmospheric N_2 that is then given to the plant in form of ammonia (NH_3^-) (Long, 1989; Fisher and Long, 1992). As a result of these previous studies, it has been seen that in terms of the obtained yields, rhizobium tends to diminish the crop production in comparison to synthetic nitrogen fertilizers (Olivera *et al.*, 2004; Karak and Bhattacharyya, 2011; Ackerman *et al.*, 2013) while its use on soil for common bean reduces about 19% per ha of the environmental burden when mineral N fertilization is replaced (Araujo, Urbano and González-Andrés, 2020).

To summarize the state of the art in nutrient recovery for mineral fertilizer substitution, the above-mentioned studies have shown that struvite can reduce and substitute a significant amount of P fertilizer while recovering great amounts of phosphate nutrients from WWTP. On the other hand, rhizobium can reduce the need for nitrogen mineral fertilizers, only partially reducing the environmental impact associated to the use of fossil-dependent mineral fertilizers. However, no study has attempted to use both struvite and rhizobium to completely avoid the application of nitrogen and phosphorous fertilizer, thereby reducing environmental impacts even further.

This paper aims to fill this gap by exploring the feasibility and environmental impact of applying struvite combined with rhizobium inoculation as alternative fertilizers of a UA system. To do so, we use the Life Cycle Assessment (LCA) to quantify the environmental impacts of a common bean crop in which the seeds were inoculated with soil bacteria rhizobium and different quantities of struvite were applied. The bush bean *Phaseolus vulgaris* "Pongo" was used in the experiments due to previous tryouts showing a good production in the perlite substrate as well as for being a highly consumed leguminous crop in Spain. The objective is to show the benefits and costs of this fertilizer alternative when compared to mineral fertilizer, and provide knowledge towards reducing resource extraction for Urban Agriculture (UA). The article is organized as follows:

After an introduction describing the state of the art and the motivation behind this work (1), we proceed to describe the experiments, the various sources for nutrients, and the LCA methodology, including system boundaries and functional unit in section (2). Next we present the obtained results for the crop production as well as the nutrient balance and environmental performance (3). Finally our findings are further discussed in section (4) leading to the final conclusions of this work (5).

2. Materials & Methods:

2.1 Description of integrated Rooftop Greenhouse (i-RTG)

The experiments were conducted in the greenhouse laboratories for UA located on the integrated Rooftop Greenhouse Laboratory (i-RTG-Lab) of the Environmental Science and technology building (ICTA-UAB) located in the Universitat Autònoma de Barcelona campus (UTM: 42º29'24" E, 45º94'36" N). The irrigation system is hydroponic on substrate with primary the use of rainwater. The 900m^2 rainwater harvesting system (RWHS) is included in the building structure as well as a 100m^3 storage tank located underground from which the water for irrigation is pumped to the cropping sites. The building structure and its year-round production have been previously analyzed to identify the environmental impact reduction due to the connectivity and synergy between the greenhouse and the building (Sanjuan-Delmás *et al.*, 2018). This building has two greenhouse laboratories for UA on the fourth floor, where this experiment was conducted. The beans were planted on the South- West facing laboratory (Urban Agriculture Laboratory 2) with a total area of 122.8m^2 as can be seen on the plant layout shown in figure 1.

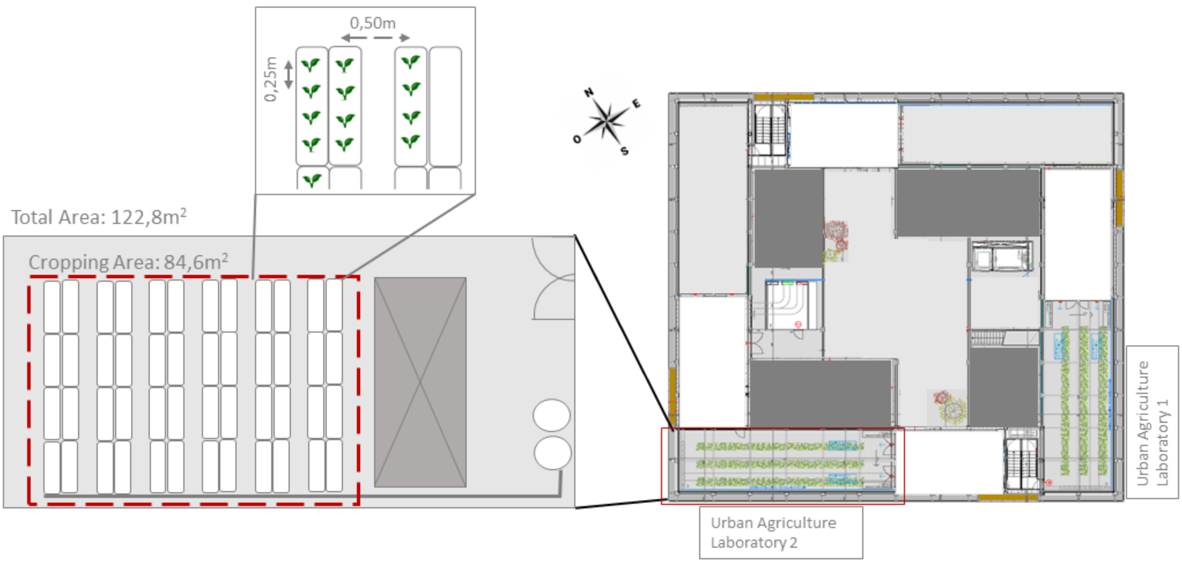


Figure 1 Experimental Layout of the experiment in the i-RTG.

Several sensors were used to monitor temperature (T107 Campbell Scientific) and relative humidity of the i-RTG cropping areas (Table 1 in the supplementary information). Irrigation water, water drainage, electric conductivity and pH for each irrigation line were measured three times a week.

2.2 Plant materials and growth conditions

The seedlings were obtained from a nursery, where the seeds were inoculated with the rhizobium mix and transported to the i-RTG 10 days after planting. The production system is soilless with perlite substrate and nutrient solution given through the 2L/h drip irrigation system. The cropping area was arranged in twelve rows with four 40L perlite bags each (Figure 1). Four bean plants were planted in each

1m long perlite bag, making a total of 192 plants, divided in three treatments (64 per treatment). The plantation frame was $0.125m^2$ within a total cropping area of $84.6m^2$. The irrigation was set 4 times a day for 3 minutes giving a total amount of 400ml per day for each plant.

Two experiments were performed. The first experiment took place in 2019, starting on the 16th of January and ending on the 10th of April. The second experiment took place in 2020, and the plants were transplanted on the 13th of February and the experiment was finalized on the 7th of May, lasting each one a total of 84 days. We used different concentrations of struvite during the two experiments to determine the N and P assimilation rates and how the yield was affected. In 2019, the bean plants were treated with 2g of struvite (1.02 m*mol* of P; 0.46 m*mol* of N) per plant (SR2) and 5g of struvite (2.57 m*mol* of P; 1.15 m*mol* of N) per plant (SR5). In 2020, we incremented the amount of struvite to 10g (5.13mmol of P; 2.32mmol of N) per plant (SR10), and 20g of struvite (10.27mmol of P; 4.64mmol of N) per plant (SR20). The inoculation was made prior to their sowing, embedding the bean seeds in the commercial liquid rhizobium mixture before planting. We performed a control experiment both years that was fertilized with a conventional nutritional solution, with zero struvite and without inoculation.

The struvite granules were placed close to the root area to ensure a better absorption by the plant. To avoid possible runoff of struvite granules a 1L bag with small holes for water drainage, was placed around the root area to retain the crystalline granules close to the plant. The granulated urine derived struvite was given directly to the plants rhizosphere after transplanting them into the integrated greenhouse.

2.2.1 Commercial inorganic fertilizer

Two nutrient solutions were made for both campaigns, one standard full nutrient solution (NS) with nitrogen, phosphorous and magnesium and a second solution deficient in nitrogen, phosphorous and magnesium with a higher content in K_2SO_4 to avoid potassium as a limiting factor. All nutrients were mixed into a concentrated solution stored in 50L tanks, further diluted with rainwater when irrigated in a ratio of 1:100 (NS:Rainwater).

Table 1: Nutrient solution content, Control NS as the full NS for all control plants and the Mg, P, N-free NS for all treated plants with rhizobia inoculation and struvite.

Nutrients applied	*Control NS*	*Mg, P, N-free NS*
KPO_4H_2	136 mg/L	---
KNO_3	101 mg/L	---
K_2SO_4	217 mg/L	435 mg/L
$Ca(NO_3)_2$	164 mg/	---
$CaCl_2$	111 mg/L	111 mg/L
$Mg(NO_3)_2$	148.3 mg/L	---
Hortilon	0.1 mg/L	0.1 mg/L

Sequestrene	0.1 mg/L	0.1 mg/L

2.2.2 Commercial Rhizobium inoculant

The inoculant used for this experiment was obtained through a company based in Karlsruhe, Germany, nadicom GmbH. This 1L liquid product contained a mixture of two rhizobia strains, *Rhizobium phaseoli* and *Rhizobium giardinii*, that were directly applied on the bean seeds (except for the Control) before planting and again 5 days after transplanting to the ICTA RTG- Lab. The manufacture and transport of this commercial product was not included in the LCA as an input for our alternatively fertilized crops, since the production impact has been considered minimal.

2.2.3 Urine derived struvite

The struvite used for the experiment was urine derived, obtained from a wastewater treatment plant (WWTP) in Denmark. The plant recovers struvite from the digestate flow through the addition of reagents to reach stoichiometric levels that trigger struvite precipitation. The obtained struvite ($Mg(NH_4)PO_4{\cdot}6H_2O$) has a composition conformed by 12.5% w/w phosphorous; 5.7% w/w nitrogen and 9.9% w/w magnesium and a granule size of 1 to 3mm. The heavy metal content in struvite from different origins and production systems has been analyzed and set at levels under the European threshold, also ranging far below the amount of possible impurities that can be found with the production of phosphate rock as well as untreated sewage sludge from WWTP (Bastida *et al.*, 2019).

2.3 Experimental analyses and nutrient balances

Water samples were taken from each irrigation system as well as the drained water 3 times a week. Production of the bean plants was counted and weighted. The amount of drained leachates were measured daily and sampled three times a week. The concentrations of Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, Ca^{2+}, K^+ and Mg^{2+} were measured using ionic chromatography. Additionally, the pH and EC were measured daily for both the nutrient solutions and leachate water. To reduce the possible error generated through the irrigation and sampling the generated data was adjusted to a curve. The incoming and outgoing nutrients were quantified as well as the nutrients found in the plant biomass and beans. The plant biomass was collected at the end of the experiment with a sample number of 8 plants per treatment. These samples were dried and weighted before being digested with a Single Reaction Chamber microwave with concentrated HNO_3. The digested samples where then analyzed using Optical Spectrometry (ICP-OES). The same procedure was applied to the obtained production of beans, sampled throughout the experiment. The final balance per plant was assessed with the following equation:

$$Eq\ 1: \qquad Fns + Fs + Ffix = Fp + Fb + Fpl + Fl$$

Equation 1: Fp= g nutrients in production, Fb= g nutrients in biomass, Fpl= g nutrients in perlite, Fl= g nutrients in leachates, Fns= g nutrients in nutrient solution, Fs= g in struvite , Ffix= g nutrients obtained through N_2 fixation

To calculate the fraction needed per plant to close the balance, the following equation was used (Eq2):

$$\text{Eq 2:}\quad \text{Balance \%} = 100 * \frac{Fp}{(Fns + Fs + Ffix)} + \frac{Fb}{(Fns + Fs + Ffix)} + \frac{\text{Fpl}}{(Fns + Fs + Ffix)} + \frac{\text{Fl}}{(Fns + Fs + Ffix)}$$

Equation 2: Fp= g nutrients in production, Fb= g nutrients in biomass, Fpl= g nutrients in perlite, Fl= g nutrients in leachates, Fns= g nutrients in nutrient solution, Fs= g in struvite, Ffix= g nutrients obtained through N_2 fixation

The following results depict the data collected in 2019 for plant biomass, irrigation and leachate nutrient content as well as yield production and nutrient content. The 2020 study was included to provide further information on the effect of greater struvite quantities to increase the yield. Therefore, the LCA results for 2020 only defer from the 2019 inventory in the amount of struvite used as well as the yield.

Additionally an analysis to calculate the fraction of N in the biomass obtained from N_2 fixation was made, using an elemental analyzer- isotopic ratio mass spectrometer (EA-IRMS; Thermo Fisher Scientific), attaining the $\delta^{15}N$ values (in ‰) for our treatments SR2, SR5 and control as well as our alternative fertilizer struvite which was set in 7.1‰. Contributions from each source (atmospheric or struvite) were then calculated with the following equation (Shearer and Kohl, 1993; Unkovich *et al.*, 2002; Arndt *et al.*, 2004), using the lowest $\delta^{15}N$ value obtained as our 'B' value (-1.16‰) (Shearer and Kohl, 1989; Peoples, Boddey and Herridge, 2002; Kermah *et al.*, 2018):

$$Eq\ 3:\qquad \%Ndfa = \frac{\delta^{15}N\ Source\ 2 - \ \delta^{15}N\ Sink}{\delta^{15}N\ Source\ 2 - 'B'value} \times 100$$

Equation 3: %Ndfa (Nitrogen derived from N2 fixation from the atmosphere), δ15N Source 2 (‰) corresponds to the δ15N value of struvite, δ15N Sink (‰) corresponds to the δ15N value from the sample, 'B' value set at -1.16‰

2.4 Life Cycle Assessment (LCA)

The LCA is a tool with a standardized methodology (ISO 2006) used to determine the environmental performance of goods in all stages of their life cycle of the four proposed treatments (SR2, SR5, SR10 and SR20) and control. The scope of the LCA study is cradle to gate of the bean production system. The functional unit (FU) chosen is 1kg of fresh beans at the collection point. The cut-off method in the Simapro software was applied which allocates the benefit of the recycled materials to the recycled products. To calculate the life cycle environmental impacts of the treatment, we used the Simapro software and the EcoInvent 3.5 attributional database. The following impact categories (IC) were selected, all from the ReCiPe (H) Midpoint method: Global warming (GW), Terrestrial acidification (TA), Freshwater eutrophication (FE), Marine Eutrophication (ME), Fossil Resource Scarcity (FRS) and Ecotoxicity (ET), which is the sum of Freshwater, Marine and Terrestrial ecotoxicities.

The system definition is illustrated by figure 2, which differentiates between the subsystems infrastructure, operation and end of life. For infrastructure, we considered the production and end of life of the greenhouse, the rainwater harvesting system and the auxiliary equipment such as pumps and fertirrigation installed and all the transportation required. All steps shown in figure 2 for raw material extraction, processing, transport to construction site, construction/ maintenance, as well as the transport to the landfill or recycling site were considered. On the operational side, the study includes the production, use and end of life, including transport, of all the resources required for the duration of the experiments (perlite substrate, fertilizers, struvite, pesticides, water, and energy). Exceptions to this are the production of nursery plants and the composting of the residual biomass as well as the rhizobium production. For the production of struvite the additional inputs for controlled precipitation were accounted, in this case the chemical inputs can be seen in the LCA inventory, which consisted in MgCl, Energy and $NaOH^-$ for 1kg of struvite as described by the technology developed by Ostara® (Amann *et al.*, 2018). The used wastewater for the struvite precipitation was not considered within the system boundaries for this study.

For the end of life subsystem of our production several assumptions were made. The remaining biomass generated in the greenhouse goes to composting as well as the used substrate after 5 years of use. The composting of the residual biomass was not considered within the system boundaries. The leachate water was discharged into the urban water cycle entering the wastewater treatment plant. All phosphates and nitrates discharged into the water are therefore considered direct emissions to water, in the case of the treatments fertilized with struvite, also as direct emissions to the air. For the system infrastructure it was considered that the RWHS as well as the Auxiliary equipment were assumed to be disposed of into the landfill. The distance to the landfill and recycling site were assumed to be 30 km from the greenhouse.

The inventory data for the infrastructure and auxiliary equipment was compiled from Sanyé *et al.*, 2012; Sanyé Mengual, 2015; Sanjuan-Delmás *et al.*, 2018; Rufí-Salís, Petit-Boix, *et al.*, 2020. For both the rainwater harvesting system (RWHS) and the i-RTG System a lifespan of 50 years was considered while the auxiliary equipment was set at 10 years, taking into account previous work by Sanjuan-Delmás *et al.*, 2018 and Rufí-Salís, Petit-Boix, *et al.*, 2020. Emission factors for N to air and N and P to water were calculated according to Llorach-Massana *et al.*, 2017. The emissions to air in struvite where calculated taking into account the emission factor of the total nitrogen and phosphorous in the applied quantity of struvite, even when not all struvite was dissolved.

For the transportation of all materials average values for the transport to markets were given. The transport to the i-RTG was then added with a distance of 50km for all pesticides, fertilizers and auxiliary equipment as well as the struvite and rhizobium applied. Transport distance of 850km was applied for the substrate bags following the methodology of Sanjuan-Delmás *et al.*, 2018. No transport of the horticultural production was considered since one of the benefits of urban agriculture is the on-site selling of the products, therefore the product procurement by the consumer is located outside our system boundaries.

The data for the operation was collected during the experiment, including the amount of fertilizers, the substrate used as well as the energy used to work the irrigation system. The energy used during the campaign was estimated by the water pumps and amount of water pumped to the greenhouse and crops.

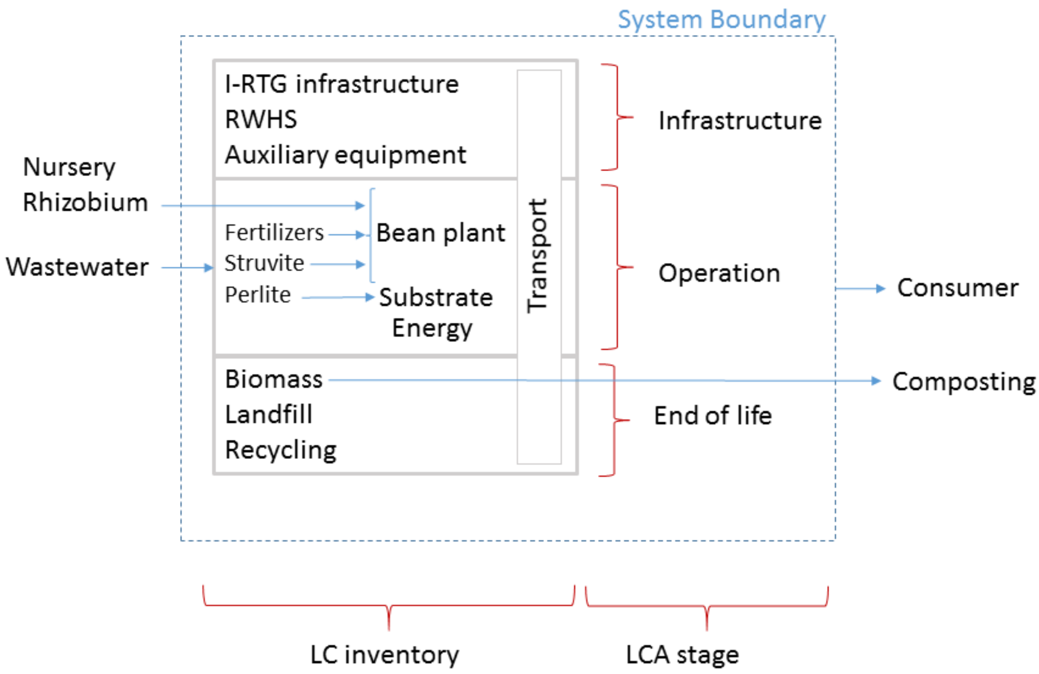


Figure 2: Representation of the LCA System boundaries of the present experiment for fresh bean production. Division between the Operation al system, the Infrastructure and the End of life subsystems. LCA inventory represented on the left and the LCA stages on the right. The system boundary defined by the dotted line delimiting within the accounted materials and stages in this study.

The full inventory is available in supporting information (Tables 2 to 6 from the supplementary information).

3 Results:

3.1 Yields and nutrient balance

The total and average-per-plant production for both campaigns (2019 and 2020) can be seen in Table 2 for each treatment. The results show that as struvite concentration is increased from SR2 to SR20, production also increases from 1899.2g to 4821.5g indicating a significant assimilation rate of P by the plants. It is crucial to point out that the two controls differ greatly as well from one campaign to the other. Since the campaign 2020 began one month after the 2019 campaign, we can consider the climatic conditions as an explanation for greater productions, taking into account that minimal temperatures were higher in 2020 (by more than 6ºC), as well as the average temperature throughout the experiment (Table 1 supplementary information). These conditions would enable a greater and faster production of flowers and an earlier bean growth. The first harvest made in 2019 was 49 days after transplanting the bean plants into the greenhouse while in 2020 the harvest began 39 days after transplanting (Figures 1 and 2 Supplementary information). Due to the different climatic conditions and resulting productions the control treatment for both campaigns has to be considered as a reference.

*Table 2: Production for campaigns 2019 and 2020 for all treatments * Difference to control = percentage in relation to control as 100%*

PRODUCTION	2019			2020		
TREATMENT	SR2	SR5	Control 1	SR10	SR20	Control 2
TOTAL	1899.2g	2375.6g	4726.7g	3542.2g	4821.5g	8198.4g
AVERAGE PER PLANT	59.3g	74.2g	147.7g	110.7g	150.6g	256.2g
DIF TO CONTROL*	40.2%	50.3%	100%	43.2%	58.8%	100%

The observed productions do not reach more than 60% of the achieved production in the control treatment, staying between 40 to 60%, regardless of the increased struvite quantities.

3.2 Nutrient fluxes

The obtained nutrient balance can be seen in table 3. Here the nutrient content for Nitrogen, Phosphorous, Magnesium, Potassium, Sulfate and Calcium in the incoming nutrient solution can be observed, as well as the outgoing fluxes of production (bean pods), biomass (leaves, stems, roots), perlite and leachates. In the case of nitrogen the fixated N_2 is also taken into account as seen in figure 3.

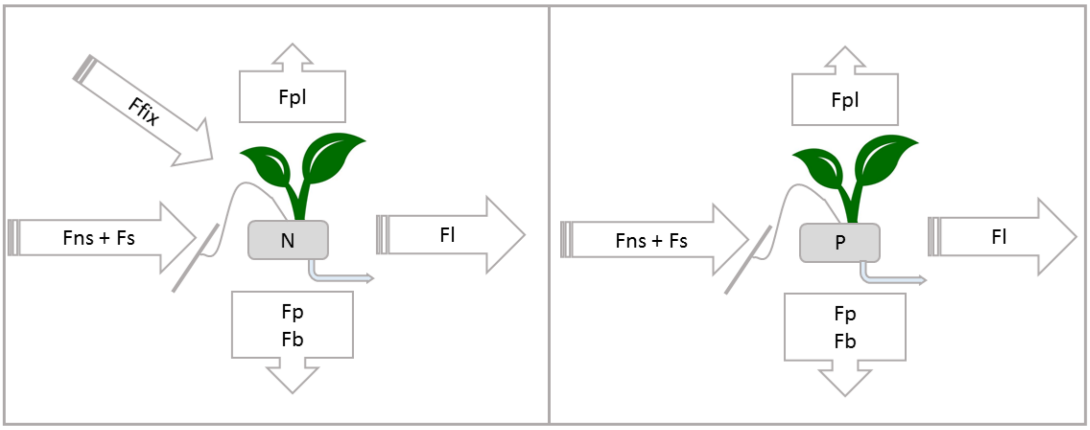


Figure 3: Nutrient flow representation for N (left) and P (right). Fp= g nutrients in production, Fb= g nutrients in biomass, Fpl= g nutrients in perlite, Fl= g nutrients in leachates, Fns= g nutrients in nutrient solution, Fs= g in struvite, Ffix= g nutrients obtained through N_2 fixation

The obtained results for the fixed g of N where achieved thanks to previous studies with isotopic N15 analyses where the percentages of fixation for SR2, SR5 and the 2019 control where obtained. The average percentage of fixed nitrogen for SR2 was 82% while for SR5 it was 72% and 16% for the control of the total N found in production and biomass. This fixed Nitrogen was further given as an additional inflow.

The balance for nutrients N, H, K, and Ca is close to 100%, indicating that the inflows can be traced almost entirely in the different outflows. On the other hand, there are losses of P and Mg in the SR2 and SR5 treatments, which are unaccounted for in the mass balance showing percentages under 50% reaching values as low as 23% for the P balance in SR5. We consider that the reason for such low accounting of the balance for P and Mg is the possibility of them remaining undissolved in the perlite bag. While Sanjuan-Delmas provides information of the amounts of these nutrients in fertigation that can be found in the perlite, the remaining P, Mg and N from struvite left in the perlite bag was not determined. This factor can generate uncertainty in our nutrient balance which has to be taken into account. On the other hand the N balance seems to fit the incoming and out coming flows, while the amount of the given N with the struvite is respectively lower to Mg and P and the atmospheric N_2 fixation also amounts to additional nitrogen in plant biomass and production. When regarding the percentage of dicharded P and N into the leachtes we can observe a reduction of almost threefold in N when comparing the control (23%) to both treatments (SR2 with 8% and SR5 with 9%) and double (SR2 with 17%) or even fourfold (SR5 with 9%) in P compared to the control (37%).

Table 3: Nutrient balance per plant for N, P, Mg, K, S and Ca Fp= g nutrients in production, Fb= g nutrients in biomass, Fpl= g nutrients in perlite, Fl= g nutrients in leachates, Fns= g nutrients in nutrient solution, Fs= g in struvite , Ffix= g nutrients obtained through N_2 fixation **perlite was obtained from Sanjuan-Delmas.*

Nutrient	Treatment	Nutrient solution+ Struvite (Fns+Fs)	Atm N_2 Fix (Ffix)	Production (Fp)		Biomass (Fb)		Perlite* (Fpl)	Leachates (Fl)		Balance
		g	g	g	%	g	%	%	g	%	%
N	SR2	0.114	0.293	0.152	37%	0.204	50%	0%	0.031	8%	95%
	SR5	0.285	0.301	0.159	27%	0.262	45%	0%	0.052	9%	81%
	Control	1.271	0.123	0.376	27%	0.375	27%	6%	0.323	23%	83%
P	SR2	0.25	0	0.021	8%	0.036	14%	0%	0.041	17%	40%
	SR5	0.625	0	0.030	5%	0.057	9%	0%	0.055	9%	23%
	Control	0.740	0	0.081	11%	0.109	15%	6%	0.274	37%	69%
Mg	SR2	0.198	0	0.011	6%	0.020	10%	0%	0.096	49%	64%
	SR5	0.495	0	0.016	3%	0.050	10%	0%	0.133	27%	40%
	Control	0.461	0	0.032	7%	0.060	13%	0%	0.292	63%	83%
K	SR2	6.357	0	0.173	3%	0.270	4%	0%	4.249	67%	74%
	SR5	6.357	0	0.206	3%	0.357	6%	0%	4.128	65%	74%
	Control	4.774	0	0.576	12%	0.739	15%	0%	3.664	77%	104%
S	SR2	1.626	0	0.009	1%	0.030	2%	0%	1.420	87%	90%
	SR5	1.626	0	0.011	1%	0.040	2%	0%	1.405	86%	90%
	Control	1.611	0	0.030	2%	0.060	4%	0%	1.040	65%	70%

Ca	SR2	1.713	0	0.021 1%	0.150 9%	3%	1.126 66%	79%
	SR5	1.713	0	0.025 1%	0.195 11%	3%	1.374 80%	96%
	Control	1.719	0	0.061 4%	0.475 28%	3%	1.127 66%	100%

3.3 Environmental performance of the treatments:

The LCA impacts per functional unit (FU) are disaggregated into the life cycle stages that resulted in the highest impacts for all four treatments and controls, as shown in figure 4. Since the controls resulted in higher yields, consequently the impacts are reduced considerably in all categories except for Freshwater and Marine Eutrophication (FE, ME).

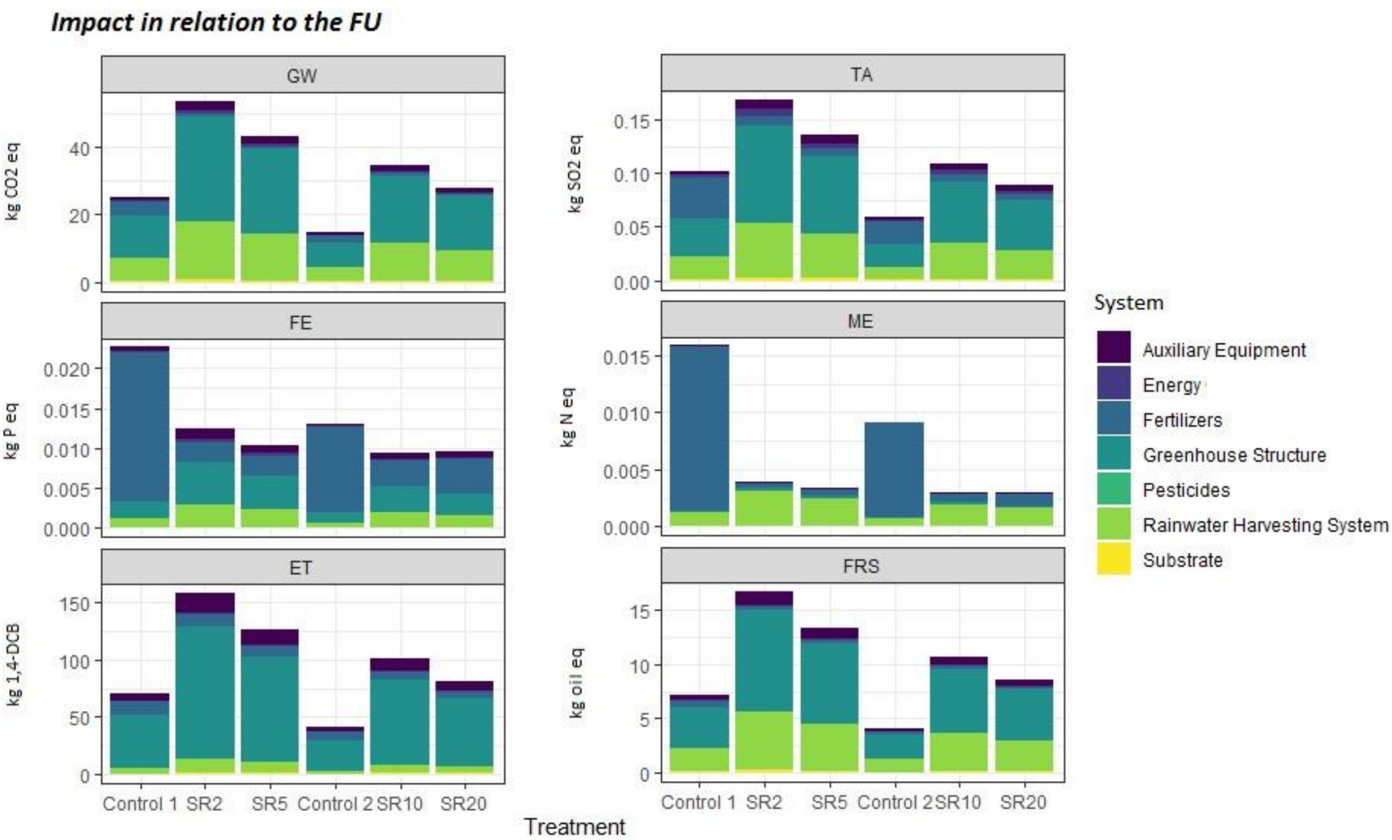


Figure 4: Total System and operation impacts in relation to the Functional Unit.

Within each impact category we can clearly state that the greenhouse structure and the rainwater harvesting system account for most of the generated impact especially in GW, TA and FRS. This can be due to the large transport distances, the processing and construction of larger amounts of materials like aluminum and steel.

While the auxiliary equipment and fertilizers seem to have lower impacts in most categories, the implication of the latter in the ME and FE categories is of great importance for the control treatments where a full nutrient solution was used. Even when the emissions to air and water of struvite were taken into account, the reduction in these two categories for treatments SR2 and SR5 is especially clear.

While the higher production in the control treatment reduces impacts of the RTG- infrastructure and RWHS, the impact generated by the fertilizers is still greater in all IC for the control despite the higher yields. The percentage contribution of the accounted system stages can be further observed in figure 5. The reduction of the impact generated by the alternative fertilizer can be seen when comparing the smaller percentages for fertilization in the treatments SR2 and SR5 to the control treatments 1 and 2.

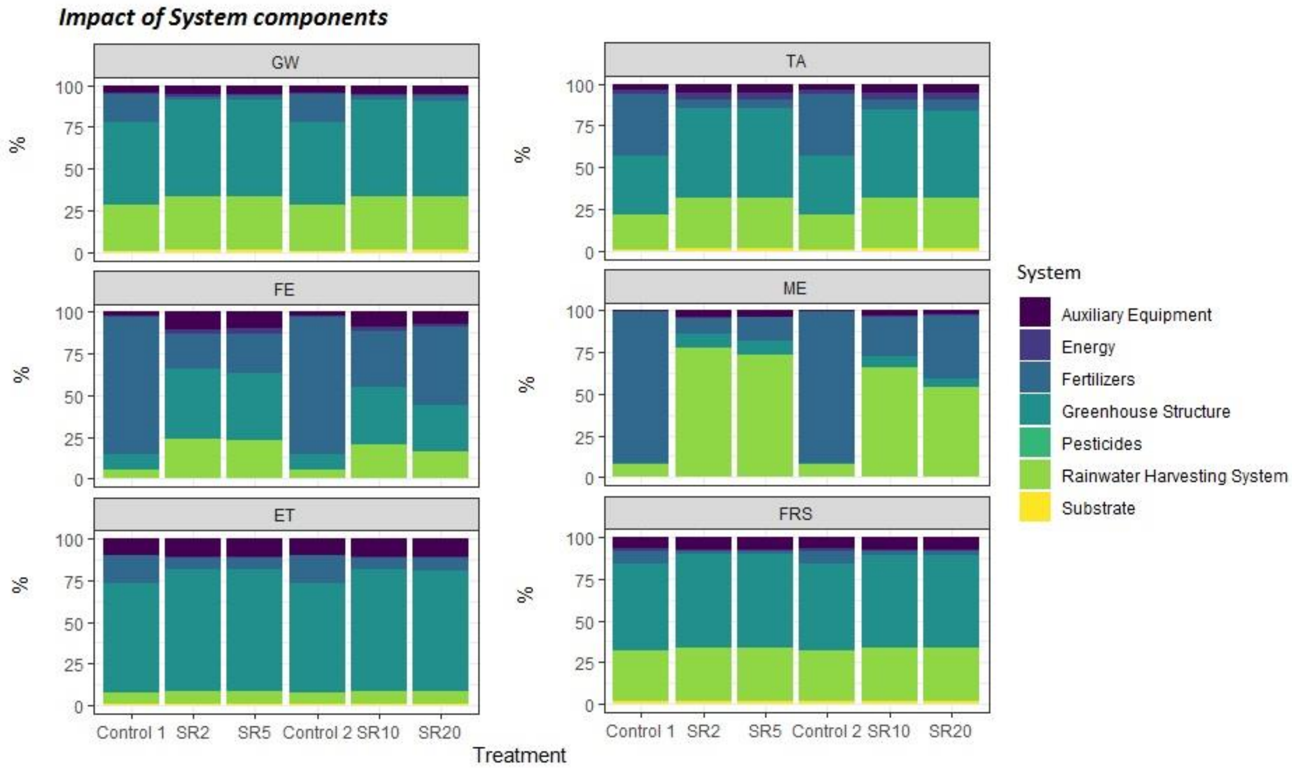


Figure 5: Impact of System components (%)

When adding the percentages corresponding to the infrastructure (RTG-structure, RWHS, auxiliary equipment) and operation (energy, fertilizers, pesticides, and substrate) stages of production for each impact category, the shift of the weight of impact contribution with the alternative fertilization can be seen (table 4).

Table 4: Emission origin in our experiment from Infrastructure or Operation of the System in each impact category (IC)

TREATMENT	SR2		SR5		CONTROL	
IC	Infrastructure	Operation	Infrastructure	Operation	Infrastructure	Operation
GW	96%	4%	95%	5%	82%	18%
TA	90%	10%	90%	10%	60%	40%
FE	76%	24%	73%	27%	17%	83%
ME	97%	3%	89%	11%	9%	91%
ET	91%	9%	91%	9%	82%	18%
FRS	95%	5%	95%	5%	89%	11%
TREATMENT	SR10		SR20		CONTROL 2	
IC	Infrastructure	Operation	Infrastructure	Operation	Infrastructure	Operation
GW	95%	5%	95%	5%	82%	18%
TA	89%	11%	88%	12%	60%	40%
FE	64%	36%	51%	49%	17%	83%
ME	76%	24%	62%	38%	9%	91%
ET	91%	9%	91%	9%	82%	18%
FRS	95%	5%	95%	5%	89%	11%

The change in the fertilization mainly generates a shift in the eutrophication impact categories (FE and ME) which reach up to more than 80% of the total impact of the operation in the control treatment whilst staying under 30% in both treatment SR2 and SR5. It is also worth mentioning that overall the change in the fertilization has an effect on all categories, shifting the weight of the impact from the operational phase to the infrastructure when comparing the control treatment to the SR2 and SR5 productions.

Due to the great percentage taken up by the greenhouse structure and rainwater harvesting system, the contribution of the operational side of the bean production is overshadowed. Since the infrastructure remains the same for all treatments and is highly specific to this particular site, it was excluded from consideration in figure 6 for a better exploration of the effects of the substituting fertilizer (Figure 3 Supplementary information for Environmental performance of the operation System in % per IC).

When observing figure 6 the applied fertilization appears to be the main cause for emissions in all IC. While yield is smaller in all four struvite and rhizobium treatments, emissions remain lower than controls 1 and 2 in most categories except ecotoxicity and fossil resource scarcity. Still it is worth mentioning that the reason for a higher emission in these two categories is not bound to fertilization but due to an increase of the weight of substrate and energy in the operation impact.

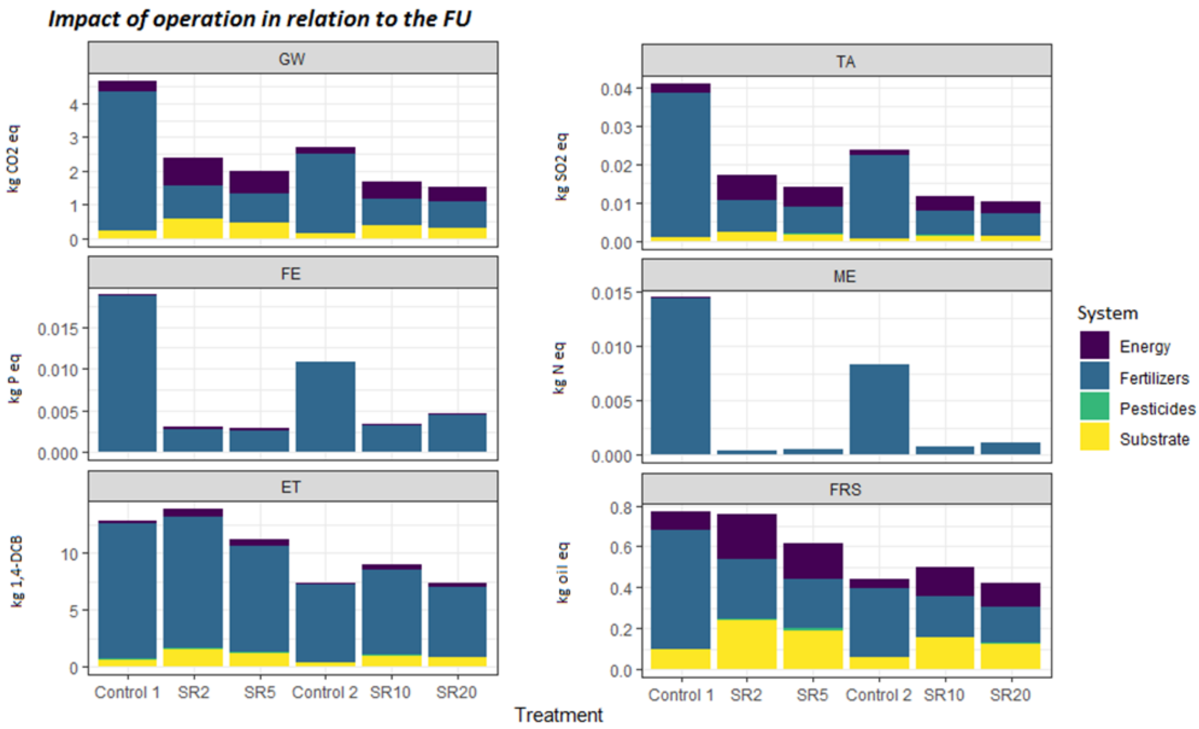


Figure 6: Impact of the production operation in relation to the Functional Unit

While emissions are mostly lower in the four alternative fertilizer treatments, especially in SR20 in GW, TA, ET and FRS, we can observe a slight increase with greater amounts of struvite in both categories FE and ME. To further understand the changes in emissions bound to fertilization, figure 7 depicts all accounted factors considered in the LCA for the fertilization.

When observing the fertilization emission in figure 7, a great reduction can be seen in all IC in the four treatments in comparison with the respective control treatments. Great impact reductions are made due to the reduced emissions to air and water (as seen in TA, GW, FE and ME) and transport of fertilizers (as seen in ET, FRS and again GW). Here we can appreciate that the slight increase of impact seen for treatment SR20 achieved in categories FE and ME is due to water emissions increase due to greater struvite quantities.

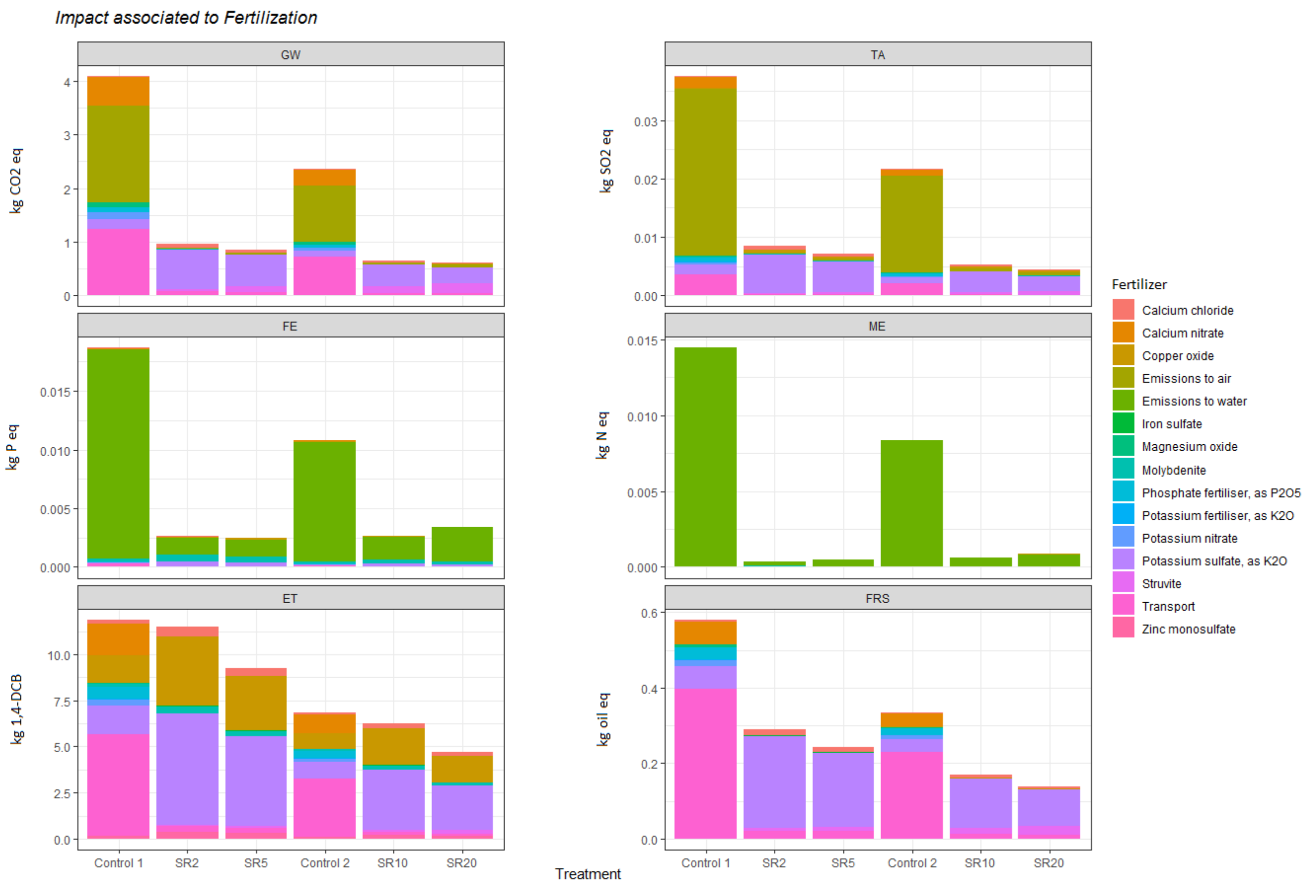


Figure 7: Impact associated to the treatment fertilization in relation to the Functional Unit

4 Discussion:

The life cycle assessment performed on the bean production experiments in soilless substrate fertilized with struvite and rhizobium has shown that there are significant benefits in terms of eutrophication. These findings confirm studies of other authors such as (Sanjuan-Delmás *et al.*, 2018; Rufí-Salís, Calvo, *et al.*, 2020) in which fertilization has been deemed as a major contributor to the environmental footprint of urban agriculture. However, because the yield is lower than that of the conventional mineral fertilizer, the impacts associated to the infrastructure required for the fertilizer substitution increase. Fertilization has shown to be of great importance in the impacts regarding our bean production. The sole removal of the nitrogen, phosphorous and magnesium from the fertigation has shifted the weight of the emissions from the operational part to the infrastructure in a drastic manner. These emission reductions not only affected the expected IC (ME and FE) but all due to their transport (for GW, ET and FRS) and the emissions to Air (for TA) as seen in figure 7. While this information depicts great flaws in the implementation of such production systems it also gives a great chance for improvement. While the application of struvite has shown to fulfill the entire cycle of the crop with some yield reduction its production and transport do not affect the given IC to a greater extent. The accumulation of all three fertilizers (P, N and Mg) in one and the possibility of its local generation and application can considerably improve the operational footprint of our agricultural systems. While the struvite recovery technology developed by Ostara® and used for this experiment requires inputs of MgCl, energy and NaOH-, has been seen to have lower environmental impacts compared to other processes (Rufí-Salís, Brunnhofer, *et al.*, 2020) further advancements are being made on the use of saltwater, to further reduce the use of chemical resources and potentially lowering its environmental footprint (Martínez-Blanco *et al.*, 2014; Hasler *et al.*, 2015; Amann *et al.*, 2018).

On the other hand, as we have seen in the nutrient fluxes for the SR2 and SR5 treatments, the percentages of the balances for P and Mg remain low. The previously described slow dissolution of the struvite fertilizer (Bhuiyan, Mavinic and Beckie, 2007; Degryse, Baird, Rodrigo C da Silva, *et al.*, 2017) has been identified as the reason for the lower balance percentages, leaving struvite in the bag that has still not been diluted. This dissolution could be remedied with a lower pH in the irrigation as well as an increase of the irrigation points inside the bag. The location of the struvite itself with regard to the root area has also been regarded as relevant for its plant uptake (Degryse, Baird, Rodrigo C. da Silva, *et al.*, 2017). On the other hand the remains of struvite inside the bag can favor the reuse and recycling of the perlite bag for a less P and Mg demanding crop in the case of treatments SR2 and even a second production of beans like in the case of SR5. A second bean production without the addition of struvite would even further reduce the needed inputs and its operational footprint.

The use of alternative fertilizers like struvite avoids the consumption of mineral or synthetic fertilizers (Lam, Zlatanović and van der Hoek, 2020) described as fertilizer offset accounting. Then environmental benefit of the use of struvite should not only be accounted in the moment of its use (emissions to air and water) and transport but in the avoided production of N, P, and Mg fertilizer. Even further, the environmental benefit of the removal of these nutrients from urban waterbodies should be taken into account as well. As described before, the generation of struvite has requirements but removes potential water and air emissions from WWTP (Ishii and Boyer, 2015a; Igos *et al.*, 2017; Lam, Zlatanović and van der Hoek, 2020). While this last benefit has not been taken into account for this study, the further use of struvite as fertilizer and the consequent fertilization offset accounting have, and can be well observed in these results with the emission reductions in almost all IC.

The yield reduction in all treatments compared to the respective controls has a great impact on the production footprint. While a higher production has been reported with a greater application of struvite, a limitation to reach greater yields still remains. Plausible explanations for these losses are the reduced struvite dissolution (Degryse, Baird, Rodrigo C. da Silva, *et al.*, 2017), the higher P requirement due to the rhizobia symbiosis(Long, 1989; Olivera *et al.*, 2004) or a possible electrochemical imbalance causing a reduced uptake of cations? in the root area as described by Kontopoulou *et al.*, 2015.

While the yield reduction remains unclear, its impact on the environmental performance is quick to be identified in the obtained results. The loss of production increases the environmental footprint of production, reaching higher emissions than the control treatments (especially when infrastructure is considered), even with no use of N, P and Mg fertilizer.

A higher yield without the additional use of these fertilizers would decrease the impact of our production, which begs the question as up to what yield loss percentage we can afford and still remain more sustainable than the control treatment.

To answer this question the control (2019) yield was regarded as our hypothetical 100% yield and therefore a scenario with 0% yield reduction. From here on the emissions for all IC were calculated with a yield loss of 10% to 60%. The values used for the 60% and 50% yield loss where directly taken from the SR2 and SR5 treatments respectively, since the obtained yields corresponded to the simulated yield losses. The emissions were also derived from the SR2 and SR5 treatments, since no additional fertilizer were supposed to be added.

Figures 8 and 9 depict these scenarios for the yield reduction impact in infrastructure and operation and only operation respectively.

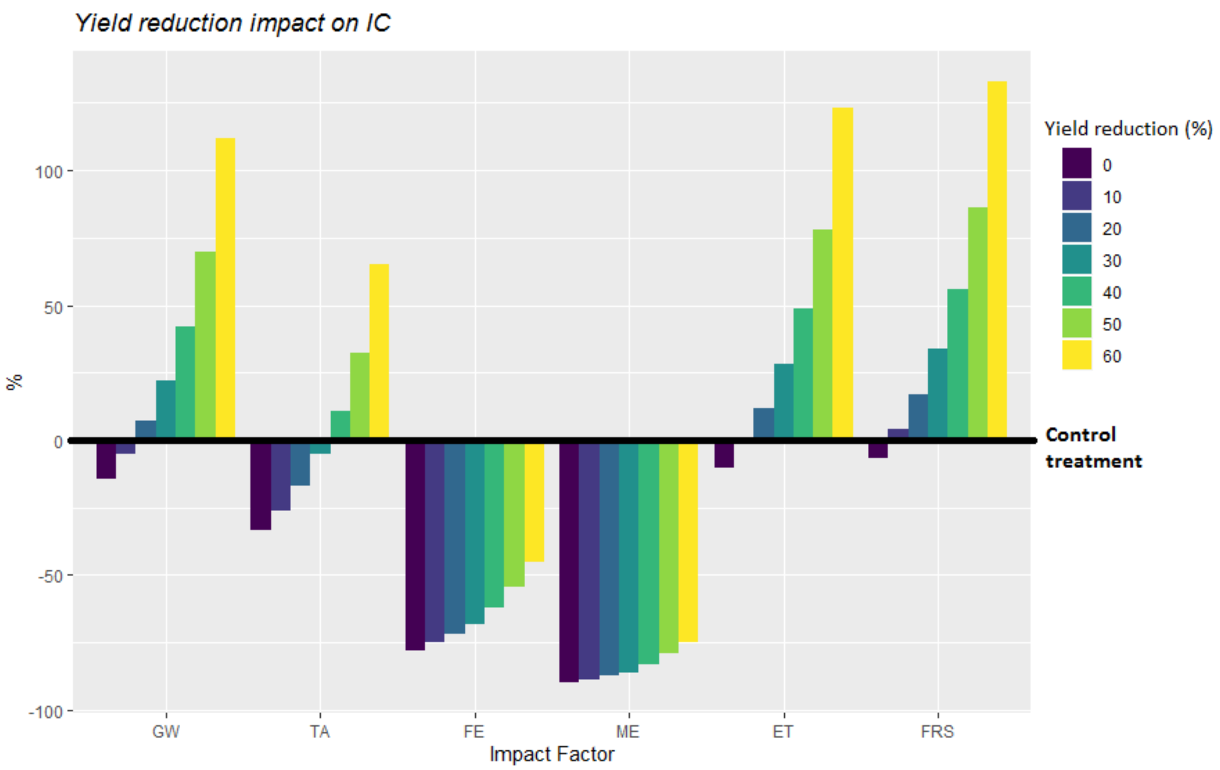


Figure 8: Yield reduction (in %) impact on IC (in %) for infrastructure and operation systems.

The control treatment line in both figures 8 and 9 is where the baseline from the control treatment was set. Above this control line the emissions are increased with regard to the control (in %), while below this line the impacts are decreased.

When considering the infrastructure and operation we can observe that no yield can be lost in order to bring all IC under the control treatment line. While FE and ME are well below the baseline other IC like TA start to decrease at 30% yield loss and below.

In the case of figure 9, when infrastructure is not considered, a 50% yield loss can occur and still decrease all emissions in all IC.

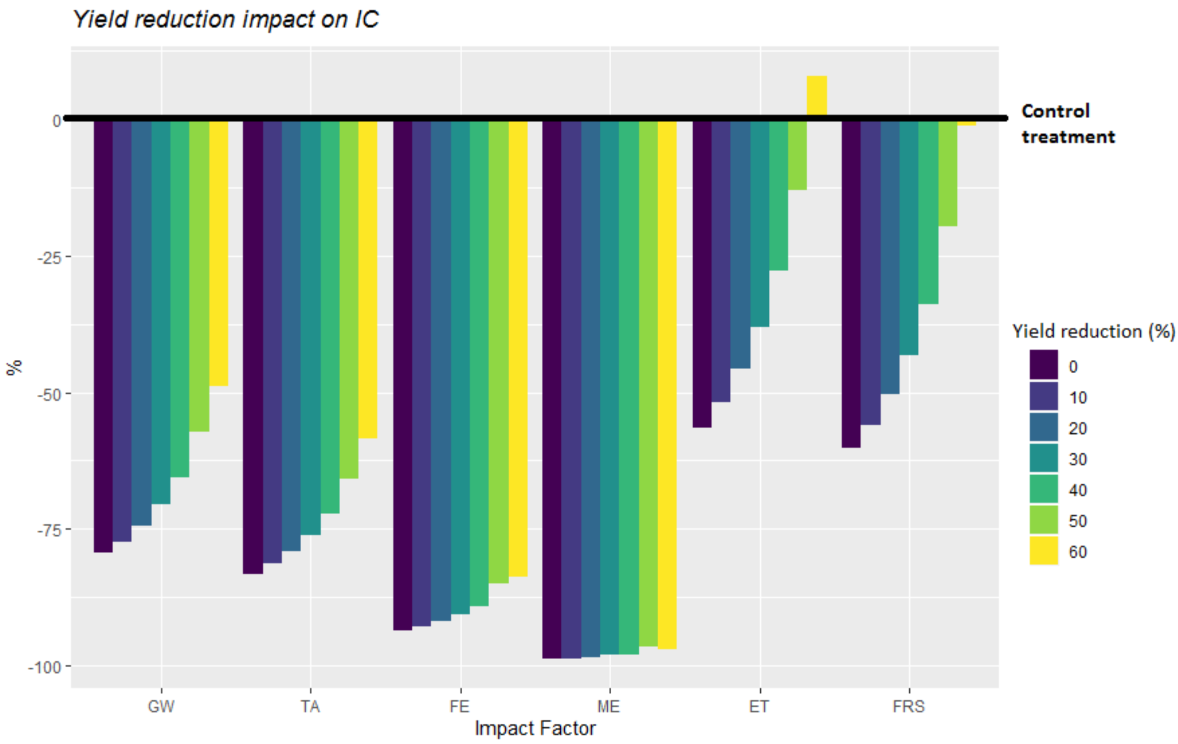


Figure 9: Yield reduction (in %) impact on IC (in %) for operation system

The importance of reducing mineral or synthetic fertilizer to avoid emission in all IC has been regarded throughout the experiment, although the importance of maintaining production levels while using alternative fertilization methods has been laid out clearly. While impact categories like FE and ME are greatly decreased in all scenarios, the capacity to affect other categories in a significant way can only be achieved with low yield reductions. Especially when considering urban agriculture, the production system might entail more complex infrastructure (rooftop greenhouse, indoor agriculture), leaving reduced margins of yield loss.

5 Conclusions:

The present work aimed to study the feasibility of bean production with the use of alternative fertilizing methods of struvite and the inoculation of rhizobium bacteria. It also aimed to analyze the environmental impact reduction obtained due to this change in fertilization and the effect of potential yield losses. To this purpose, two experiments with different struvite quantities were made and a quantification of the environmental impact using life cycle assessment as our tool. Three main conclusions can be drawn from this study:

Firstly, the total reduction of nitrogen and phosphorous mineral and synthetic fertilizers for vegetable production has been shown to be viable with the use of the recycled slow-releasing fertilizer struvite and the bacterial inoculation with rhizobium strains. Although a yield reduction in all cases was observed compromising its efficiency to reduce the environmental impact in all IC except FE and ME.

Secondly, the use of struvite and rhizobium inoculation reduced emissions in all IC mainly due to transport and emissions to air and water. The struvite, being available in all WWTP installations can be obtained with no great environmental cost while reducing transport of three separate minerals (N, P and Mg).

Thirdly, the complexity of the infrastructure and operational inputs will increase the environmental impact in all IC, as well as the yield loss. Only the reduction of yield loss up to 0% can equal the environmental impact of the control treatment in all selected categories when the infrastructure is considered. Without the infrastructure the margins for yield loss can range up to 50% staying below the control treatment. Therefore we consider crucial to reduce infrastructure complexity in the prospect of urban agriculture as well as the reduction of mineral and synthetic fertilizers to truly reduce potential environmental impacts.

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Verónica Arcas-Pilz: Conceptualization, Methodology, Formal analysis, Data curation, Writing- Original draft; **Martí Rufí-Salís.**: Methodology, Data curation, Writing - review & editing; **Felipe Parada**: Methodology, Writing - review & editing; **Xavier Gabarrell**: Conceptualization, Methodology, Writing - review & editing, Supervision; **Gara Villalba:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

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