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Use of a Non-Ionic Water Surfactant in Lettuce Fertigation for Optimizing Water Use, Improving Nutrient Use Efficiency, and Increasing Crop Quality

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Abstract: The use of water surfactants in fertigation constitutes a viable approach to increase soil wetting, potentially improving crop nutrient uptake and quality. An in-field demonstration test was carried out by applying an innovative, eco-friendly, non-ionic surfactant to fertigation water in *Lactuca sativa* (var. Iceberg) production to increase nutrient use efficiency and improve the crop's access to water. A non-ionic methyl-oxirane surfactant (methyl-oxirane + 2-methyl-oxirane) was added at an increasing rate to the fertigation solution (Hoagland). Upon harvesting, the main growth and nutritional parameters were determined on the aboveground and belowground portions of the lettuce. Leaf nitrate content, water, and nitrogen use efficiency were recorded; the relationship of lettuce aboveground dry biomass with nutrient uptake was evaluated using vectorial analysis; and ultrastructural analysis of lettuce roots was performed by scanning electron microscopy. The surfactant, applied by fertigation at the rate of 1.0 mL × $\mathrm{L_{Hoagland}}^{-1}$, improved crop P, K, Mn, and Fe use efficiency. When applied by fertigation, although the surfactant did not increase the water use efficiency index, it induced a significant decrease of the specific leaf water content (−8.8%) and an increase of the leaf area (+13.3%). By comparison with the recent literature, we inferred a positive physiological response by more expanded and less thick leaves in lettuce, likely by the optimization of the crop water and nutrient root uptakes mediated by the abundant but shortest lateral roots. This finding corresponded to the lowest leaf nitrate content, indicating an improvement of the lettuce quality without losing the crop yield.

Keywords: water infiltration; nitrogen use efficiency; specific leaf water content; vegetable production; soil; yield

1. Introduction

Under the effects of climate change, high temperatures and extreme weather events occurring within a short period of time, especially in areas with poor vegetation cover or bare soil, lead to soil erosion, aridity, and loss of organic matter. As a result, the supply of adequate water and nutrients to crops is becoming an issue [1]. In the Mediterranean region, water scarcity and the increasing pressure over water availability coming from other productive sectors urge farmers to find alternative solutions to improve the water and nutrient uptake by crops, exploiting all technical innovations available on the market [2]. Moreover, even if excessive fertilization does encourage great yields, at the same time it results in water and soil pollution; the possibility of reducing nutrient inputs and improving crop nutrient use efficiency may instead reduce the amount of fertilizers employed by farmers.

Among other solutions, the use of water surfactants in fertigation could constitute a viable approach to increase crops nutrient use efficiency and quality [3] by reducing soil water repellency [4], increasing water infiltration rate, and limiting runoff in Pacific Northwest soils [5]. This would indicate an improvement of the water use efficiency, guaranteeing that an adequate amount of nutrients reaches the crops.

The adjuvants comprise a broad range of substances, of which solvents and surfactants are the major types [6]. In particular, surfactants are organic molecules, containing both hydrophobic and hydrophilic groups, which act at the interface between two different phases by lowering the surface tension of a liquid. Among them, the non-ionic, modified methyl co-polymers constitute a class of wetting agents where the molecular structure was modified by replacing terminal hydroxyl groups with methyl caps. As –OH is a hydrophilic group while $-CH_3$ is typically hydrophobic, these molecular changes modify the hydrophilic properties of the surfactant, thus modulating its water repellency and wettability [7].

Their mode of action allows liquid solutions to penetrate and wet agricultural soils more easily, potentially improving water use efficiency and crop quality [3,8]. This behavior has been demonstrated for hydrophobic soils [6,9] and in recent years has received attention for hydrophilic soils [10–12]. The properties of surfactants seem to be correlated with their intrinsic strength and the concentration of the solution [11]. Moreover, it has been demonstrated that their characteristic properties markedly change when reaching a critical concentration of the surfactant solutions (CMC, critical micelle concentration) [9]. At the CMC, surface active ions or molecules in the solution (such as the available nutrients in soil) associate to form larger units (micelles) in the presence of the surfactant. The CMC corresponds to the concentration of surfactants above which micelles form, constituting a key parameter able to interact with their super-spreading effect [9–11]. The possibility of using this kind of surfactant as adjuvants in the irrigation water to be applied on agricultural land has been poorly investigated so far, with contrasting results. Their potential effects on crops uptake and growth is currently being explored [13], and a first theoretical model of their mechanism of action in soil was recently provided [11]. McCauley [14] evaluated the effect of a non-ionic surfactant on soybean (*Glycine max* L.), and found that yield increased with surfactant application to the irrigation water. Other authors reported no effects on plant growth after surfactant application by fertigation to corn (*Zea mays* L.), soybean, wheat (*Triticum aestivum* L.), and potato [15–17]. Preliminary research has shown that non-ionic surfactants added to the irrigation water may increase root growth [3,18]. Another study on turf grass revealed neither positive nor negative effects on macronutrient and micronutrient uptake due to fertigation with surfactant application [19]. Similar results were obtained by Banks [20], who observed no consistent effects on nutrient uptake after surfactant application to corn in different soils. Surfactant application at planting is considered a management technique that may reduce nitrate leaching from potato fields [13]. As far as the nitrogen is concerned, Arriaga et al. [21] found that the use of a non-ionic surfactant may reduce nitrogen leaching and improve nitrogen utilization in potatoes. A recent work showed that the application of a non-ionic surfactant to irrigation water in corn production under a Mediterranean climate gave a net increase of water use efficiency and, in parallel, a high corn yield and dry matter. This testified an undoubted economic advantage to farmers by saving water and reducing fertilizer inputs; in fact, even if the surfactant application increased the irrigation costs by 4.7%, it increased the profit by 19.7% [22].

The potential advantages for water conservation deriving from surfactants application to soil could be profitably exploited by Mediterranean farmers involved in vegetable production, which suffer due to scarce rainfalls in spring and summer cropping seasons [22–24]. Since in many countries, such as Italy, Greece, and Spain, vegetable production represents a relevant commercial sector and water availability is becoming an issue, all technical-agronomical strategies to reduce water input and improve nutrient use efficiency by increasing soil wettability are considered valuable alternatives to the indiscriminate use of such precious resources that are often dispersed inefficiently in the environment [25]. At the moment, very little information is available about the potential synergistic or antagonistic effects of surfactants addition to irrigation water on broad-leaved vegetable production as well as its interaction with mineral fertilization. A recent experimental trial on lettuce after the addition of a non-ionic surfactant to the irrigation water in the absence of fertilization gave positive results on water and soil nutrient uptake [11]. However, it is not yet clear if and how these types of non-ionic surfactants, added to mineral fertilizers in fertigation, are able to interact and thus modulate the translocation of the macro, meso, and micronutrients from the root to the vegetable leaves.

The objective of the present research was to verify whether the addition of an eco-friendly, non-ionic surfactant to fertigation could improve the nutrient use efficiency and quality of lettuce via the optimization of crop water uptake. The final purpose was to reduce the water supply in broad-leaved vegetable production while guaranteeing the same crop yield. Since different surfactants were available on the market, our choice in surfactant selection was guided by its molecular structure, chemical properties, and biodegradability in the environment [26,27].

2. Materials and Methods

Water surfactant characteristics and use—The tested surfactant (methyl oxirane surfactant, MOS) was a non-ionic fluid material, composed of 80% *w*/*w* methyl-oxirane and 20% *w*/*w* of 2-methyloxirane, and produced by a patented industrial process. The chemical structure of this surfactant consisted in a hydrophilic head group (2-methyl-oxirane) and hydrophobic tails $[–O–Si–(CH_3)_3]_n$, which gave it a typical water repelling property. It was defined as an eco-friendly compound, since its final biodegradability was >80% under aerobic conditions in 28 days, on the basis of the application of the eco-toxicity testing methods reported in the Council Regulation (EC) No 440/2008 (EC method C.4-D, 440/2008/EEC) and the Organisation for Economic Co-operation and Development (OECD) guideline 301 F (1992) [28,29]. In order to define the best application dose of MOS as a fertigation surfactant in crop production, it was added to the fertigation solution at two different doses: $0.2\ mL_{MOS} \times L_{Hoagland}^{-1}$ (F S0.2 solution) or $1.0\ mL_{MOS} \times L_{Hoagland}^{-1}$ (F S1.0 solution). The control treatment (F CNT) was fertilized with the same fertigation solution without the addition of MOS.

Experimental site and design—A one-year in-field demonstration test was carried out on a broad-leaved vegetable crop to evaluate the influence of MOS used in fertigation on crop nutrient uptake by changes in water use by the crop. The research was conducted in open field, at the experimental site of the Council for Agricultural Research and Economics, Research Center for Agriculture and Environment (CREA-AA) in Rome (Central Italy), (N 41°53′7.475″; E 12°29′43.464″ 42 m a.s.l.) with a typical thermo-Mediterranean climate. The absolute annual temperatures ranged between 0 °C in winter and 40 °C in summer. The field test lasted 40 days, from April to May 2015; in this period, temperatures ranged between 16 and 28 °C and no rainfalls were recorded during the trial.

Soil was characterized in relation to texture, pH, organic C (C_{org} %), total nitrogen (N_{tot}%), cation exchange capacity (CEC, meq 100 g^{-1}), organic matter (%), available phosphorous (P_{Olsen}, mg kg^{-1}), total potassium (K_2O, mg kg^{-1}), exchangeable calcium (Ca, meq 100 g^{-1}), potassium (K, meq 100 g^{-1}), sodium (Na, meq 100 g^{-1}), magnesium (Mg, meq 100 g^{-1}), and cadmium (Cd), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), and zinc (Zn) (mg kg^{-1}). The soil chemical-physical properties are reported in Table 1.

Table 1. Main soil physicochemical parameters [30]. CEC: cation exchange capacity.

Soil Parameter			
Silt (%)	47.6	Ca (meq 100 g^{-1})	24.3
Sand (%)	24.4	K (meq 100 g^{-1})	1.3
Loam (%)	28.0	Na (meq 100 g^{-1})	3.2
pH	7.6	Mg (meq 100 g^{-1})	0.7
$C_{organic}$ (%)	1.21	Cd (mg kg^{-1})	<0.05
N_{tot} (%)	0.12	Cu (mg kg^{-1})	1.0
CEC (meq 100 g^{-1})	29	Fe (mg kg^{-1})	401.1
Organic matter (%)	1.79	Ni (mg kg^{-1})	0.6
P_{Olsen} (mg kg^{-1})	25.2	Pb (mg kg^{-1})	2.1
K_2O (mg kg^{-1})	598.1	Zn (mg kg^{-1})	1.3

Lettuce (*Lactuca sativa* var. "Iceberg") seedlings, grown in 2 cm × 2 cm × 4 cm of 60% perlite + 40% peat growing media, were transplanted in soil at about a height of 6 cm (three fully expanded leaves). In a randomized three-block designed system of 18 m^2, 60 plants per block (20 plants per treatment) were transplanted, for a total of 180 plants. The treatments were the fertigated control (F CNT) and two different doses of surfactant (F S0.2 and F S1.0) added to the fertigation solution. An additional non-fertilized control (NF CNT) (20 plants per block) was additionally considered only for calculations on the Nitrogen Uptake Efficiency: NUpE = $(N_F - N_{NF})/N_F$, where N_{NF} was the N uptake of the unfertilized plot (NF CNT) and N_F were those of fertilized plots. Data on NF CNT were reported exclusively for calculating the NUpE, since the aim of this research was to evaluate the effect of MOS application on nutrient availability supplied by fertigation.

Fertigation was performed by applying a half-strength (50%) Hoagland solution to the lettuce, in order to emphasize the effect of the surfactant addition in suboptimal nutrient supply. The 50% Hoagland solution was prepared by diluting 0.25 g L^{-1} KNO_3, 0.068 g L^{-1} KH_2PO_4, 0.59 g L^{-1} $Ca(NO_3)_2{\cdot}4\ H_2O$, and 0.25 g L^{-1} $MgSO_4{\cdot}7\ H_2O$ in 1 L of distilled water. Drip-fertigation was carried out by administering 50 mL per plant of the nutrient solution after 1, 3, 8, and 16 days from transplanting (with a total of 200 mL/plant of fertilized solution). The Hoagland solution was added to the fertilized control (F CNT). To supply the surfactant by fertigation, MOS was added in the Hoagland solution at a concentration of 0.2 $mL_{MOS} \times {L_{Hoagland}}^{-1}$ for the treatments F S0.2 and 1.0 $mL_{MOS} \times {L_{Hoagland}}^{-1}$ for the treatments F S1.0. During experimental trial, each plant was irrigated with 50 mL of distilled water on alternate days, strictly avoiding subirrigation. During the whole lettuce cropping cycle, the total amount of water was 600 mL water per plant.

At harvest, multiple parameters were determined for the crop: (i) crop growth and leaf water content; (ii) crop water and nutrient use efficiency; (iii) plant root growth and morphology.

Crop growth and leaf water content—In order to evaluate the effect of the application of MOS by fertigation on crop growth and water uptake by lettuce, at harvest, five plants/treatment/block, for a total of 15 plants/treatment, were collected and separated into aboveground and belowground portions, then dried in a forced-air oven at 80 °C for 72 h in order to determine the dry biomass and leaf water content. The following data were collected, separately for the aboveground and belowground lettuce: fresh (FW) and dry (DW) weight (g $plant^{-1}$); leaf area (LA, cm^2 $plant^{-1}$), dry matter (DM, total dry weight/fresh weight); number of leaves (N. leaves $plant^{-1}$); specific leaf fresh (LFW) and dry (LDW) weight (mg cm^{-2}), specific leaf water content (SLWC, as LFW − LDW, mg cm^{-2}); root fresh (RF, g $plant^{-1}$) and dry weight (RD, g $plant^{-1}$), root dry matter (RDM), and root to shoot ratio (RS). Leaf area (LA) was measured using an electronic area meter (LI-COR Model 3100, Delta-T Devices Ltd., Cambridge, UK).

Crop water and nutrient use efficiency—To assess the potential benefit of the surfactant treatments, the water use efficiency (WUE, in g L^{-1}) was calculated on lettuce fresh (FWUE) and dry (DWUE) biomass, as the ratio between the aboveground fresh (FW) or dry (DW) weight (g) and the applied amount of irrigation water [11,22].

Crop nutrient use efficiency was evaluated by applying the vector analysis to all nutrients (N, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, B) [31] and by calculating the nitrogen uptake efficiency (NUpE) [32]. For the mineral analysis, dried leaf tissues, taken at the end of the experiment, were ground separately in a Wiley mill (20-mesh screen). Then, 1.0 g of the dried plant tissues were analyzed for the following elemental content: P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, B. The related concentrations were determined by dry ashing at 400 °C for 24 h, dissolving the ash in 1:25 HCl, and assaying the solution obtained using an inductively coupled plasma emission spectrophotometer (ICP-AES Thermo Optek, Milano, Italy) [33]. The N content (%) of both the aboveground lettuce (N_P) and the belowground portions (N_R) was determined on a dry-weight basis, using a nitrogen analyzer (FP-528; Leco Corp-USA) to calculate the lettuce N uptake. The leaf nitrate concentration (NO_3, in mg/kg) was determined by a nitrate test (116995–Reflectoquant, Merck, Darmstadt, Germany). On the basis of the amount of N supplied by the half-strength Hoagland solution, equal to 21 mg of N per plant, the N uptake efficiency (NUpE) was calculated as the ratio: $((U_F - U_{NF})/N_F)$, where N_F was the N supplied by fertigation, U_F the N uptake when N_F was given, and U_{NF} the N uptake in the control plot that was not fertilized (NF CNT) [26].

Root growth and morphology—On the basis of previous research, where the role of different agronomic strategies on roots development in horticultural and tree crops was evaluated by scanning electron microscopy (SEM) [34,35], in the present work the effect of MOS on lettuce root morphology was evaluated by visual observation and SEM analysis, by selecting three roots fragments per plant and collecting three plants per treatment. In particular, secondary lateral roots were cut with a razor blade from 5 mm to 15 mm from the root tip to assess the potential effect induced by the surfactant on the turgidity of the meristematic cells. The fresh root fragments were observed by SEM (Carl Zeiss A.G., Oberkochen-Germany) under variable pressure equipped with a tungsten (W) electron source, using the backscattered electrons detector (SEM-BSE), which is able to improve the resolution so as to optimize the visualization of the biological ultra-structural root morphology.

Statistical analysis—All data were statistically analyzed by ANOVA with post hoc Tukey's HSD test or Duncan's multiple-range test for means comparison using the SPSS software package (IBM Corp., Armonk, NY, USA). We applied Tukey's HSD test (checking that the model assumptions were met), as it exhibited a greater power than the other tests under most circumstances (e.g., Bonferroni tends to lack power overcorrecting for Type I error). The method of Duncan, which is less conservative, was applied as alternative test when the data showed some tendency to be not significant with Tukey's test (i.e., $p \approx 0.05$), since Tukey pays the price of a greatly increased Type II error rate. Bi-dimensional vector analysis was then applied for the simultaneous comparison of plant growth and nutrient content [3,31]. Under vector analysis, changes in nutrient content, nutrient concentration, and dry weight were plotted as vectors in a bi-dimensional graph, with each point representing the combination of these three parameters within four Cartesian subplots. Nutrient content obtained under the different treatments were compared after normalization, while changes in dry weight and concentration were plotted with curved content isoclines included for interpretation. Dry weights were displayed in relation to the nutrient content of plant tissue; the abscissas represented the dry weight (x-axis) and the ordinates represented the nutrient concentration (y-axis) [3,31].

3. Results

Crop growth and leaf water content—Results showed that fertigation with MOS application at both the doses did not affect the considered aboveground (FW, DW, DM, N leaves, LA) and belowground (RF, RD, RDM, R:S) parameters (Table 2 and Figure 1), with the exception of LFW, which was lower in presence of the surfactant at both the concentrations ($p \leq 0.05$, Figure 1). MOS application also determined a slight, but not significant increase of the aboveground DM values (Table 2).

Table 2. Surfactant and fertigation effects on aboveground and belowground biometric parameters: fresh (FW) and dry (DW) weight (g plant^{-1}), dry matter (DM), number of leaves (N. leaves plant^{-1}), specific dry (LDW) weight (leaf weight/leaf area, mg cm^{-2}), root fresh (RF) and dry (RD) weight (g plant^{-1}), root dry matter (RDM), root:shoot ratio (RS). F CNT = fertigation control, F S0.2 and F S1.0 = surfactant application by fertigation at 0.2 mL$_{MOS}$ × L$_{Hoagland}$$^{-1}$ and 1.0 mL$_{MOS}$ × L$_{Hoagland}$$^{-1}$, respectively. Data are reported as mean ± standard error (SE). Significant differences at $p < 0.05$ (Tukey's HSD post hoc test). [a]: *, **, *** = significant at $p \leq 0.05$, 0.01, and 0.001, respectively; NS = not significant.

Treatment	Aboveground Biometric Parameters									
	FW (g)		DW (g)		DM (%)		N. Leaves		LDW (mg/cm^2)	
		SE		SE		SE		SE		SE
F CNT	15.58	1.37	1.02	0.08	6.63	0.30	14.00	0.70	3.10	0.19
F S0.2	11.91	1.37	0.89	0.07	6.89	0.30	13.60	0.70	3.01	0.19
F S1.0	13.56	1.23	1.01	0.08	7.09	0.33	14.20	0.70	2.98	0.17
Significance [a]	NS		NS		NS		NS		NS	
Treatment	**Belowground Biometric Parameters**									
	RF (g)		**RD (g)**		**RDM (%)**		**RS**			
		SE		SE		SE		SE		
F CNT	4.13	0.38	0.36	0.03	90.96	10.95	0.34	0.06		
F S0.2	3.20	0.38	0.27	0.03	84.91	10.95	0.28	0.06		
F S1.0	3.67	0.38	0.29	0.03	85.85	10.95	0.32	0.06		
Significance [a]	NS		NS		NS		NS			

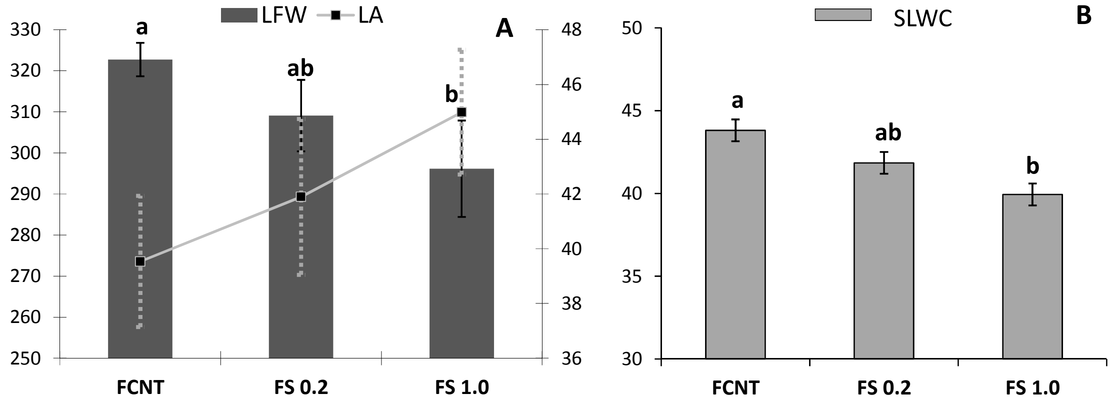

Figure 1. (**A**) Specific leaves fresh weight (LFW, mg cm^{-2}), leaf area (LA, cm^2 plant^{-1}); (**B**) specific leaf water content (SLWC, mg cm^{-2}). F CNT = fertigation control, F S0.2 and F S1.0 = surfactant application by fertigation at 0.2 mL$_{MOS}$ × L$_{Hoagland}$$^{-1}$ and 1.0 mL$_{MOS}$ × L$_{Hoagland}$$^{-1}$, respectively. Different letters represent significant differences, mean separation at $p < 0.05$ with Tukey's HSD post hoc test.

The LFW, which expresses the fresh weight of the surface unit of the lettuce leaf (cm^2), was significantly higher ($p = 0.0012$) in the control (46.9 mg cm^{-2}) with respect to that recorded after MOS application by fertigation at 1.0 mL L^{-1} (42.9 mg cm^{-2}). The increasing trend of LA (+13.3% at 1.0 mL$_{MOS}$ × L$_{Hoagland}$$^{-1}$, $p = 0.094$, Figure 1A) that was recorded for MOS-fertilized lettuce seem to suggest that the lower specific fresh weight of MOS-fertilized lettuce could be due to a major expansion of the leaf area, or to an increased evapotranspiration, or both (Figure 1A). Furthermore, the SLWC, i.e., the water content of the leaf surface unit, decreased significantly ($p = 0.042$, Figure 1B) at increasing doses of the MOS application, being about 7% lower in F S1.0 with respect to F CNT.

Crop water and nutrient use efficiency—Results related to the lettuce FWUE and DWUE are reported in Figure 2. The FWUE of F CNT was significantly higher than that of F S0.2, but it did not differ from that of F S1.0; otherwise, the DWUE was the same in F CNT and F S1.0, and again the lowest under F S0.2 treatment.

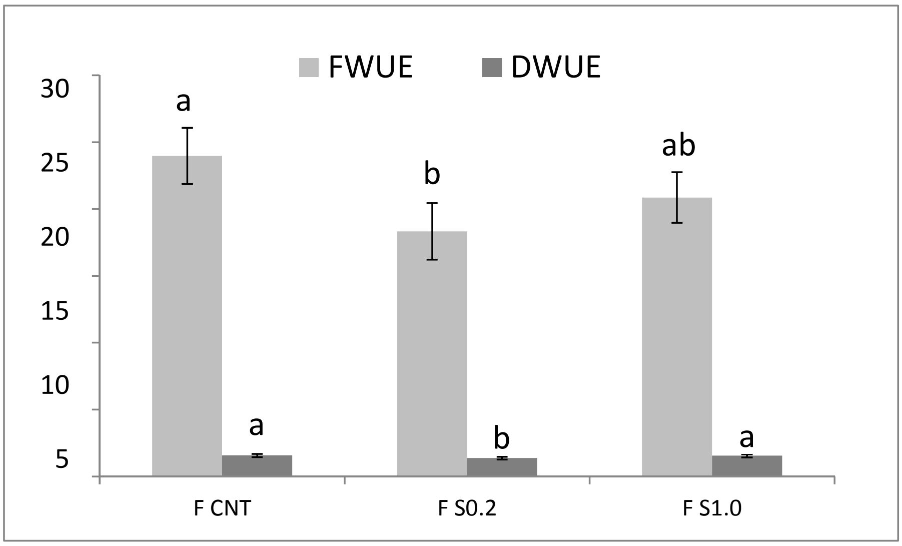

Figure 2. Water use efficiency for lettuce fresh (FWUE, g L^{-1}) and dry (DWUE, g L^{-1}) yield. F CNT = fertigation control, F S0.2 and F S1.0 = surfactant application by fertigation at 0.2 $mL_{MOS} \times L_{Hoagland}{}^{-1}$ and 1.0 $mL_{MOS} \times L_{Hoagland}{}^{-1}$, respectively. Error bars represent standard error. Different letters represent significant differences, mean separation at $p < 0.05$ with Tukey's HSD.

Regarding the nutrient uptake of lettuce, the concentrations of macro, meso, and microelements in leaves, as affected by MOS addition by fertigation, are reported in Table 3.

Table 3. Surfactant effects on macro (g kg^{-1}) and microelements (mg kg^{-1}) concentration of lettuce leaves: F S0.2 and F S1.0 = surfactant application by fertigation at 0.2 $mL_{MOS} \times L_{Hoagland}{}^{-1}$ and 1.0 $mL_{MOS} \times L_{Hoagland}{}^{-1}$, respectively; F CNT = fertigation only. Data are reported as mean ± standard error (SE). Different letters represent significant differences across surfactant treatments, mean separation at $p < 0.05$ with Duncan's multiple-range test. [a] *, **, *** = significant at $p \leq 0.05$, 0.01, and 0.001, respectively; NS = not significant.

Treatment	Macro and Mesonutrients (g kg^{-1})											
	P		K		Ca		Mg		Na		Fe	
F CNT	2.28 a	±0.12	44.36	±2.06	4.45	±0.34	1.08	±0.08	1.07	±0.08	0.09	±0.03
F S0.2	1.79 b	±0.12	40.66	±2.06	3.73	±0.30	1.20	±0.08	0.95	±0.08	0.06	±0.03
F S1.0	1.82 b	±0.15	40.49	±2.06	4.31	±0.30	1.20	±0.08	0.97	±0.08	0.07	±0.03
Significance [a]	*		NS		NS		NS		NS		NS	

Treatment	Micronutrients (mg kg^{-1})							
	B		Cu		Mn		Zn	
F CNT	11.50	±0.72	0.77 b	±0.30	62.13 a	±5.50	14.97	±1.15
F S0.2	9.76	±0.72	1.51 ab	±0.30	43.50 b	±5.50	14.34	±1.03
F S1.0	10.74	±0.72	1.99 a	±0.27	53.09 ab	±5.50	14.95	±1.03
Significance [a]	NS		*		*		NS	

Significant differences were found in the elemental concentration of lettuce leaves for P, Mn, and Cu. The P concentration decreased from 2.2 g kg^{-1} in the untreated lettuce (F CNT) to 1.8 g kg $^{-1}$ in the MOS-fertilized lettuce (Table 3). Likewise, Mn decreased from 62.13 (F CNT) to 53.09 (F S0.2) and 43.50 mg kg^{-1} (F S1.0). In contrast, Cu concentrations significantly increased from 0.77 mg kg^{-1} in F CNT to 1.51 and 1.99 mg kg^{-1} when the surfactant was administered at increasing doses by fertigation.

The bi-dimensional vector analysis, which allows for the simultaneous comparison of plant growth (i.e., lettuce aboveground dry weight) and nutrient content [3,11,31] is reported in Figure 3, showing the effect of MOS application by fertigation on lettuce nutrient use efficiency.

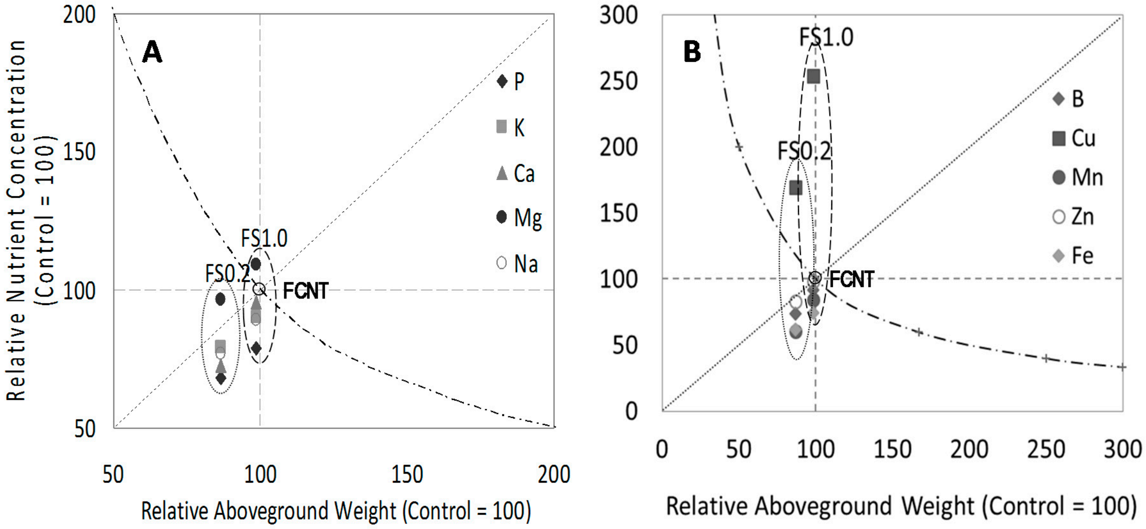

Figure 3. Vector analysis of aboveground macro (P, K) and meso (Ca, Mg, Fe, Na) nutrient (**A**), and micronutrient (B, Cu, Mn, Zn) (**B**) contents in lettuce. Each colored point is a vector, where plant aboveground weight is the x-value and the relative concentration of each nutrient is the y-value, under F S0.2 (dotted ellipse: $0.2\ \text{mL}_{\text{MOS}} \times \text{L}_{\text{Hoagland}}{}^{-1}$) or F S1.0 (dashed ellipse: $1.0\ \text{mL}_{\text{MOS}} \times \text{L}_{\text{Hoagland}}{}^{-1}$) surfactant application by fertigation. Concentration and plant aboveground dry weight of F CNT (O) were used as reference points, normalized to 100%. The content isolines in vector nomograms represent combinations of dry weight and concentration, giving the constant contents per unit of dry weight.

When administering the surfactant by fertigation at the lowest dose ($0.2\ \text{mL L}^{-1}$), both the nutrient concentration and the total content of macro (Figure 3A) and micronutrients (Figure 3B) declined, with an insignificant decrease in plant dry weight when compared to the control, giving an indication of a lowered nutrient availability that may compromise the lettuce yield. On the contrary, at the highest surfactant dose ($1.0\ \text{mL L}^{-1}$), even if the total content of macro, meso (Figure 3A), and micronutrients such as Mn and Fe (Figure 3B) decreased, no appreciable decrease in lettuce dry weight was found. At the highest rate of surfactant, the decline in P and K uptake were not a limiting factor for lettuce growth. Different behavior was noticed for Cu, which significantly increased in both of the surfactant treatments, regardless of the crop growth response.

In relation to nitrogen uptake and use efficiency, the lettuce N content of the whole plant and, separately, of the aboveground and belowground portions are reported in Table 4, together with nitrate content and NUpE, as affected by MOS applications by fertigation at both doses.

Table 4. Surfactant effects on nitrogen uptake of lettuce: F S0.2 and F S1.0 = surfactant application by fertigation at 0.2 $mL_{MOS} \times L_{Hoagland}{}^{-1}$ and 1.0 $mL_{MOS} \times L_{Hoagland}{}^{-1}$, respectively; F CNT = fertigation only. N_{TOT} = lettuce total N content, mg/kg; NO_3 = aboveground lettuce nitrate content, %; N_P = aboveground lettuce N content, %; N_R = root lettuce N content, %; and NUpE (nitrogen uptake efficiency). Data are reported as mean ± standard error (SE). Statistical analysis performed only on fertilized treatments. For fertilized treatment only: different letters represent significant differences across surfactant treatments, mean separation at $p < 0.05$ with Duncan's multiple-range test. [a]: *, **, *** = significant at $p \leq 0.05$, 0.01, and 0.001, respectively; NS = not significant.

Treatment	**Nitrogen Crop Uptake**								**Nitrogen Use**	
	Lettuce		**Plant**				**Root**		**NupE** [c]	
	N_{TOT}%		**NO_3 (mg/kg)**		**N_P%**		**N_R%**			
F CNT	1.63 ab	±0.07	21.37	±4.06	1.06 ab	±0.05	0.55	±0.090	0.46	±0.183
F S0.2	1.55 b	±0.15	21.77	±2.04	1.01 b	±0.05	0.56	±0.106	0.49	±0.106
F S1.0	1.83 a	±0.13	17.31	±2.97	1.17 a	±0.08	0.61	±0.093	0.56	±0.044
Significance [a]	**		NS		**		NS		NS	
NF CNT [b]	**0.83**	**±0.11**	**19.88**	**±4.08**	**0.78**	**±0.06**	**0.39**	**±0.094**	**-**	

Note: [b] = unfertilized control data for the calculation of NUpE. [c] = calculated as the ratio: $(U_F - U_{NF})/N_F$, U_{NF} being the N uptake in NF CNT unfertilized plot [32].

Overall, the uptake of N was evidently strongly influenced by MOS addition in fertigation at the highest rate of application, giving the highest nitrogen content of lettuce both for the whole plant (N_{TOT}, Table 4) and for the marketable fraction (N_P, Table 4). In contrast, we did not find significant differences for the nitrate content, even if a tendency to decrease was evident under the F S1.0 treatment. This is a positive result, since a reduction of leaf nitrates is considered a key parameter of crop safety. Similarly, the NUpE was not significantly affected by surfactant additions; nonetheless, a slight increasing trend was observed at increasing applications (Table 4). These results can be better highlighted by analyzing the lettuce N content by means of bi-dimensional vector analysis [11,36]. Actually, the NUpE indicates the N uptake from aboveground lettuce minus the N uptake from the soil, normalized against the N supplied by fertigation. In other words, it solely expresses the ability of the crop to take up N from the fertilized system. To investigate the use efficiency of the absorbed N, that is, the efficiency by which the crop was able to utilize the N taken up from the substrate (i.e., the fertilized soil) to grow, we analyzed the N content of the aboveground lettuce in the bi-dimensional vector plane. In Figure 4, the N_P content of lettuce leaves under F S0.2 and F S1.0 treatments was normalized to that of F CNT (control = 100) and then expressed in relation to the lettuce aboveground dry weight to emphasize the effect of the surfactant on fertigation. When MOS was added to the fertigation solution, the marketable lettuce showed a lower N relative to the unit weight under F S0.2, and an unchanged one under F S1.0, which indicates better nitrogen use efficiency in MOS-treated lettuce when compared to the F-CNT lettuce (Figure 4).

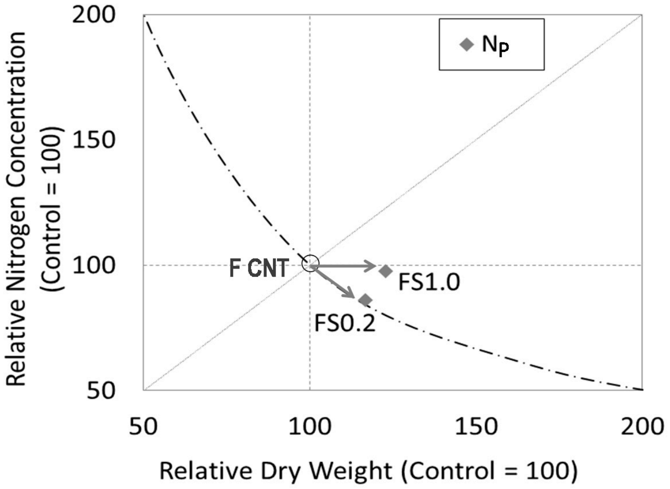

Figure 4. Vector analysis of aboveground nitrogen (N_P) content. Each colored point is a vector, where plant aboveground weight is the x-value and the relative concentration of N_P is the y-value, under F S0.2 (0.2 $mL_{MOS} \times L_{Hoagland}{}^{-1}$) or F S1.0 (1.0 $mL_{MOS} \times L_{Hoagland}{}^{-1}$) surfactant application by fertigation. Concentration and plant aboveground dry weight of F CNT (O) were used as reference points, normalized to 100%. The content isolines in vector nomograms represent combinations of dry weight and concentration, giving the constant contents per unit of dry weight.

Root growth and morphology—Root apparatus of lettuce under the different treatments was visually observed (Figure 5(A1–A3)) and then evaluated by scanning electron microscopy under variable pressure, giving a comparison of root meristematic cells (Figure 5(B1–B3)) and root apex (Figure 5(C1–C3)).

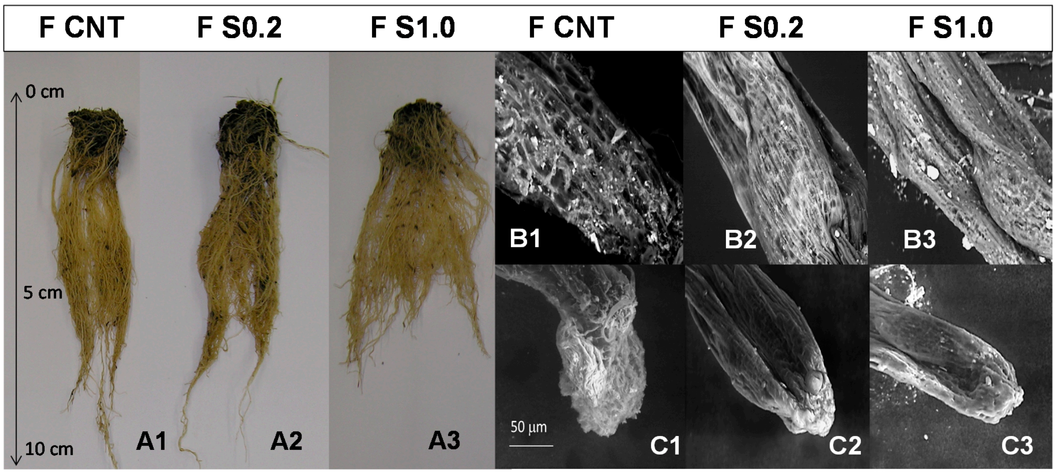

Figure 5. (**A1**–**A3**) Visual image of lettuce root apparatus. (**B1**–**B3**) Mag. 200× SEM analysis of root meristematic cells. (**C1**–**C3**) Mag. 200× SEM analysis of root apex. F CNT= control; F S0.2 and F S1.0 = MOS application by fertigation at 0.2 $mL_{MOS} \times L_{Hoagland}{}^{-1}$ and 1.0 $mL_{MOS} \times L_{Hoagland}{}^{-1}$, respectively.

Even if the RF, RD, and root DM values were the same in samples with surfactant addition with respect to F CNT (Table 2), it was evident that the root apparatus in F S0.2 and F S1.0 (Figure 5(A2,A3)) treatments were different compared to that of F CNT (Figure 5(A1)), as they were less elongated in the lettuce treated with the highest dose of surfactant. In fact, the majority of F S1.0 roots (Figure 5(A3)) had an average length of about 5.5–6.0 cm, compared to the length of 7.5–8.0 cm recorded in F CNT and F S0.2 treatments (Figure 5(A1,A2)). The SEM analysis gives evidence of a higher regularity of the meristematic cells (Figure 5(B2,B3)) and a greater turgidity of root apex (Figure 5(C2,C3)) in both the roots of F S0.2 and F S1.0 samples in comparison to the untreated control (Figure 5(B1,C1)).

4. Discussion

To evaluate the potential agronomical advantages on lettuce production deriving from the application of non-ionic surfactants by fertigation, in our demonstration tested we measured the crop yield, a set of morphological aboveground and belowground data, the FWUE/DWUE, the NUpE, and, at the end, we applied vector analysis to compare the actual aboveground lettuce dry weight and the related nutrient uptake to the theoretical crop yield and nutrient concentration (i.e., isolines). This multi-approach evaluation helped to reveal whether the application of MOS by fertigation was able to improve the lettuce quality in terms of nutritional content, ensuring the same yield, by the optimization of the crop root uptake of irrigation water.

The surfactant did not increase nor decrease the lettuce yield. In any case, due to the reduced LFW, SLWC and the increased LA, we inferred that the use of the surfactant in fertigation apparently determined a positive physiological response by inducing more expanded and thinner leaves in lettuce. In the literature, several ecophysiological studies [11,37–40] identified the leaf thickness as an index of sclerophylly that may respond to resource gradients. In fact, the lignification or suberisation of cell walls in parenchyma tissues can occur in natural environments in response to biotic and abiotic stresses, in particular to low water availability [37]. High levels of sclerophylly and increased tissue density are associated in nature with the acquisition of leaf resistance to many environmental factors, e.g., high temperature, high irradiance, and low water availability [38–40]. In our test, the recorded reduction of LFW when the surfactant was added at the highest rate could be explained by the higher leaf expansion (i.e., increased LA, +13.3% at 1.0 $\mathrm{mL_{MOS}} \times \mathrm{L_{Hoagland}}^{-1}$) and likely by the increase of evapotranspiration, with the consequent lower values of SLWC, which were −4.5% in F S0.2 and −8.8% in F S1.0 with respect to F CNT [37]. The LDW was almost constant among the considered treatments, while the DWUE did not decrease with the increase of MOS, confirming the positive effect of the surfactant in optimizing the use of water by the crop. The change in leaf morphology induced by the use of MOS in fertigation was then evaluated in relation to the lettuce nutrient uptake and root morphology. According to Scagel [31], when the content of some nutrients is lower and the plant dry weight is the same when compared to the control, the system is characterized by a higher use efficiency of such nutrients, which corresponds to lower nutritional absorption [3,11,36,41]. The slight tendency for a reduction of FW recorded in F S0.2 could be related to a reduced nutrient availability, as showed in the vector analysis. In particular, the K, Ca, and Mg content of F S0.2 (under the isoline with respect to F CNT in Figure 3), being correlated to a decrease in the aboveground dry weight, showed a less efficient use of such nutrients. On the other hands, since the lettuce N_P of F S0.2 was not located in the “deficiency” vector space (under the isoline in Figure 4), the nitrogen was evidently not a limiting factor for F S0.2 lettuce growth. When MOS was added at the highest rate, it determined the reduction of P (−20%) and, to a lesser extent, of Mn concentration (−14.6%) in lettuce tissues with respect to F CNT. This indicated that, at the rate of 1.0 mL L^{-1}, the soil nutrient availability was enough to reduce the uptake of some nutrients and increase others’ (i.e., Cu: +158%) without compromising the crop yield. While the concentration and the total content of macro and micronutrients decreased, the higher dose of MOS lead to no appreciable increases in plant weight. In this case, the nutrients uptake could be considered either luxury consumption or storage [31,42]. Since the reduction of the nutrient content in lettuce was observed only on selected nutrients, we hypothesized a different attitude of the surfactant molecules in their interaction with each ion, consequently modifying their availability to the crop into the soil in a positive (i.e., Cu) or in negative (i.e., P and Mn) way. This behavior could be due a different chemical affinity between those elements and the water surfactant molecule, which could act as a complexing agent towards certain elements in relation to their mass/charge ratio, consequently rendering them more or less available to plant roots [43].

The surfactant addition at the highest rate increased the lettuce quality. The strong decrease of leaf nitrate content (−19%) in F S1.0 with respect to F CNT, despite the corresponding increase of N_P (+10%), suggested that the water surfactant was capable to limit the nitrate accumulation in lettuce leaf. These results are partially in line with the work of Baratella et al. [3], which highlighted a dose-related detrimental effect of a non-ionic surfactant formulation (45% fatty acid ester, 45% sorbitan sesqui-octanoate, and 10% propylene glycol) as an irrigation adjuvant in the absence of N fertilization. Qiu and colleagues [44] found a highly linear relationship between nitrate accumulation and water content in different vegetable tissues, indicating that the proper soil water content in agronomic practice is essential for decreasing leaf nitrate content [45]. In our findings, N_{TOT} and N_P were higher and N_R was the same in F S1.0 with respect to F CNT; the surfactant was able to modulated lettuce N uptake in favor of the ammonium form, without incurring any negative effect to the N nutritional status of the plant. Apparently, both NO_3^- and $H_2PO_4^-$ anions exhibited reduced absorbance by F S1.0 lettuce in favor of NH_4^+ or Cu^{2+} cations. We hypothesized that their positive charges interacted with the proton-acceptor sites of the surfactant (i.e., the oxirane groups), which probably prevented their soil immobilization and made them more available to plant root uptake. In contrast, the nitrates and phosphates, as negative ions, were repelled by the surfactant, a mechanism which potentially decreased their crop availability in the short term and thus justified the decreased uptake of nitrate, P, and Mn by lettuce.

In terms of root morphology, even if no differences of RF and RD were recorded, when compared to that of F CNT, the F S1.0 root showed more organized meristem cells, a highest turgidity of the root apex, and abundant but shorter lateral roots to better intercept water and nutrients, probably localized in the upper soil layer where the surfactant was administered. Actually, at the dose of F S1.0, the root apparatus did not need to elongate more than 5.5–6.0 cm to suitability intercept the water and nutrients, while in the F CNT and F S0.2 treatments the roots grew to an 8-cm depth. Many authors argued that extensive root systems are vital when plants are grown in soils containing insufficient supplies of water or nutrients [46]. The same increased leaf expansion recorded under F S1.0 could be also correlated to a higher soil softness [47], as the consequence of the direct signaling between root and shoot was associated with the reduction of soil mechanical impedance after MOS addition [48]. It was supposed that the non-ionic surfactant, by modifying the water repellency, was able to change the water tension of the circulating solution in the soil, thus improving the wettability and spreading into the soil micropores [11–15] and reducing the depth of the soil wetted front. As a consequence, when MOS was applied by fertigation, the soil water was mainly localized in the soil volume not deeper than 5.5–6.0 cm, where lettuce roots developed. Moreover, this non-ionic surfactant led to surfactant-induced capillary pressure gradients [11] within the wetting front, which indirectly increased the lettuce roots' accessibility to nutrients [3–11].

5. Conclusions

The non-ionic surfactant MOS, applied to lettuce by fertigation at the dose of $1.0\ mL_{MOS} \times L_{Hoagland}^{-1}$, did not significantly affect the vegetable yield nor the water use efficiency index. Nonetheless, it was able to improve the nutrient use efficiency by the crop. Likely, the surfactant acted at the soil/root interface, modulating the water and nutrient uptake on the basis of their soil nutrient availability and the availability of nutrients in the soil, as well as their mutual chemical affinity with the surfactant. Since a theoretical model recently explained how non-ionic organosilicone surfactants affect soil capillary and adsorption processes in horticultural systems [11], we may correctly refer to the MOS ability to optimize lettuce water uptake and specific nutrients assimilation, in particular that of P, K, Fe, and Mn. Moreover, unexpectedly, the application of the surfactant at the highest dose strongly increased the quality of lettuce by significantly reducing the nitrate accumulation in lettuce leaves.

The obtained results should be considered to be preliminary, as they refer to an in-field demonstration test. Still, given the marketable parameters of lettuce recorded in this study, we inferred that the non-ionic surfactant, used at the highest dose, offered the possibility to: (i) guarantee the adequate rate of nutrient inputs; (ii) maintain a good standard quality of the crop in vegetable production; and (iii) potentially increase farmers' income by reducing the amount of fertigation water for vegetable production by 5–8%, without detrimental effect on crop yield. However, supplementary multi-year field trials are needed to further validate the positive feedback obtained from our demonstration experiment.

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