

Agricultural Soil as a Carbon Sink

Dott. Sergio Santangelo

Laboratori Labsan, Spoleto, Italy

***Corresponding author:** Dott. Sergio Santangelo, Laboratori Labsan, Spoleto, Italy.

Submitted: 03 March 2025 **Accepted:** 11 April 2025 **Published:** 27 June 2025

doi <https://doi.org/10.63620/MKJESSGI.2025.1010>

Citation: Santangelo. D. S. (2025). Agricultural Soil as a Carbon Sink. *J of Environ Sci Sustain & Green Innov*, 1(2), 01-10.

Abstract

CO2Fixator is the commercial name given to a microbiological preparation consisting of a mix of bacterial strains and a fungal one in determined reciprocal ratios: *Bacillus subtilis*, *Bacillus licheniformis*, *Nitrobacter*, *Lactobacillus plantarum*, *Thiobacillus denitrificans*, *Methilococcus capsulatus*; a fungal species: *Trichoderma viride*; these strains metabolize the organic soil substrate fixing carbon to the soil reducing its emission in its gaseous form of CO₂, *Trichoderma viride* promotes the development of new plants. This study is a significant step towards the control of greenhouse gas emissions from agricultural soils, CO2Fixator exploits microbial competition towards bacterial strains, already present in the soil substrate, to avoid the emission of greenhouse gases due to the fermentation of plant residues in the soil. Soil sterilization was not performed, this study intends to evaluate the effect of CO2Fixator in natural conditions. This approach offers a biological solution to mitigate the environmental impact of agriculture and other human activities. The purpose of this study is to demonstrate that sustainable agricultural practices can be developed: that reduce the carbon footprint of agriculture, that fix to the soil, in addition to greenhouse gases, also nutrients such as nitrogen by limiting its volatility, phosphorus, as well as increasing the water retention capacity or more generally the cation exchange capacity. The study has provided elements that demonstrate the increase in soil quantity and quality. The bioavailability of nitrogen and phosphorus, together with the soil's ability to provide water and the action of *Trichoderma viride*, which promotes rooting, allow to obtain a quality soil without the addition of additional substances. However, it is important to note the impoverishment of potassium that should be added to the CO2Fixator to obtain the maximum synergistic effect and support bioaugmentation. The CO2Fixator transforms agricultural soil into a Carbon Sink simultaneously improving its quality. Compared to other CO₂ capture techniques and systems, such as reforestation, algae cultivation, air filtration or conversion of CO₂ into fuel, CO2Fixator can be applied wherever there is soil, whether agricultural or not, including organic waste, sewage sludge and organic fertilizer. In the specific case of agricultural soil, bacterial digestion involves the bio-augmentation of soil components, resulting in its regeneration and making it progressively more fertile treatment after treatment.

Keywords: Soil Carbon, Climate Change Mitigation, Soil Organic Matter, Greenhouse Gases, Carbon Cycle, Soil Health.

Introduction

The regeneration of agricultural soil and its transformation into a carbon sink is an innovative and sustainable strategy to combat climate change. This approach not only improves soil fertility and its ability to produce abundant crops, but also helps reduce greenhouse gas emissions by improving soil structure and its ability to retain nutrients and water. It can also be associated with regenerative agriculture, which includes practices such as the

use of cover crops, legume cultivation and conservation agriculture, revitalizing degraded soils and promoting biodiversity [1]. These practices regenerate the soil and also create an environment more resilient to extreme weather events, improving water retention and reducing the need for irrigation. The adoption of these methods is a fundamental step towards a more ecological and productive agriculture, which respects the natural balance and contributes to the fight against global warming, which is

regularly bringing extreme weather events with enormous economic costs every year [2]. The issue of CO₂ absorption and release from cultivated fields is complex and multifactorial. As plants grow, they absorb CO₂ through the process of photosynthesis, which helps reduce the amount of CO₂ in the atmosphere. However, once crops are harvested, common agricultural practices such as burning crop residues, plowing residues into the soil, or biodigestion can actually release significant amounts of CO₂ into the atmosphere at a ratio of nearly 1:1, essentially a break-even ratio between absorbed and re-emitted CO₂ [3].

“This cycle of carbon absorption and release is a critical aspect in the sustainable management of agricultural land as a carbon sink”.

Current research explores alternative methods to manage both crop residues so that the carbon captured by plants is not released back into the atmosphere and residues of animal (e.g. manure) or human (e.g. sewage sludge) origin. An example of such an approach is "bioenergy with carbon capture and storage" (BECCS), which involves growing plants, harvesting them and then burning them to produce energy, with the CO₂ released during combustion being captured and stored [4]. Other methods include increasing soil carbon through agricultural practices that improve soil health and increase its capacity to sequester carbon such as: conservation agriculture and the use of cover crops. The aim is to develop agricultural systems that avoid releasing CO₂ and can also act as carbon sinks, thus contributing to climate change mitigation. This requires a change in traditional agricultural practices and the adoption of innovative technologies, such as CO₂Fixator, that can be scaled up. The challenge is to find a balance between food production, environmental conservation and the reduction of the climate impact of agriculture [5]. CO₂Fixator, the subject of this study, presents an approach that avoids the release of CO₂ and other polluting gases (measurable as CO₂ equivalents) and ensures the development of simpler and more sustainable agricultural practices. The agricultural sector has many margins for crop innovation and profit: the use of agricultural waste as fertilizer and the generation of carbon credits represent a significant opportunity. This practice not only reduces the need for chemical fertilizers (in fact it is already an upstream reduction of the amount of CO₂ emitted by extractive companies) but also contributes to the reduction of the carbon footprint of agriculture itself. In Italy, as in the rest of Europe, work on the definition of carbon credits and the related bank that will manage them on the market is already well underway. Farms, through the adoption of sustainable agronomic practices, can become producers of carbon credits, benefiting economically and contributing positively to the climate [6]. For example, a farmer who switches from traditional ploughing to strip tilling or no-till, who adopts burial or injection systems of slurry and digestates, who uses prescription maps to rationalize the use of mineral fertilisers and weed killers, can be assigned a certain number of carbon credits to be put on the market at a certain price. CREA (Council for Agricultural Research and Analysis of Agricultural Economics) is involved in the development of models that quantify the carbon dioxide sequestered by each cultivation activity, in various environmental conditions, and in

the certification of virtuous actions of farmers. This process of quantification and certification is essential for the creation of a reliable and transparent carbon credit market [7]. Furthermore, the Public Registry of Carbon Credits Generated on a Voluntary Basis by the National Agroforestry Sector has been established, with the aim of valorising sustainable agricultural and forestry management practices. This registry allows farmers to register the carbon credits generated and participate in a national voluntary market, in line with the provisions relating to the National Registry of Agro-Forestry Carbon Sink [8]. The use of agricultural waste as fertilizer and the generation of carbon credits are concrete examples of how agriculture can evolve towards a more sustainable and profitable model. These practices not only help reduce the use of non-renewable resources and mitigate climate change, but also offer new economic opportunities for farmers who engage in environmental sustainability paths. Even more important is the possibility for each country to become Carbon Neutral in a virtuous process where: those who emit CO₂ have the opportunity to compensate by accessing GHG carbon-sink projects implemented in their own country. Pollute here, compensate here!

Materials and Methods

The CO₂Fixator trial is a process that involves accurately measuring the levels of carbon dioxide released after crop residues are buried. Using closed chambers, a controlled environment is created to monitor emissions. These chambers, made from inverted plastic containers, are sealed to the ground to capture gases emitted by buried and decaying crop residues. Holes have been drilled into the chambers to allow gas exchange with the outside. “SCD30” CO₂ gas sensors within the chambers record gas concentrations over time, providing valuable data on the dynamics of emissions and the effectiveness of CO₂Fixator in mitigating environmental impact [9]. The sensors have been calibrated to detect gas concentrations, expressed in ppm (parts per million), from a minimum of 10ppm upwards, without limit, ensuring that the data collected is accurate and reliable. The SCD30 sensor also allows you to detect temperature and humidity parameters, useful for calibrating the sensor itself and reading the presence of CO₂ gas. The experiment was conducted in duplicate both in the agricultural field and in the laboratory. In the laboratory, the tests were performed in perforated capsules at constant temperature and humidity [10]. The soil, both for the field tests and for the laboratory tests, was prepared in such a way as to obtain a composition of 5:1 between inorganic matter and dry organic matter on all samples, corresponding to approximately 27,778 tons of dry organic matter on a hectare and approximately 100g in a container in the laboratory. Laboratory analyses are essential to understand the impact of the experiment on the soil. Through these analyses, it is possible to determine: the quantity of organic matter and whether the introduction of CO₂Fixator into the soil can improve the availability of essential nutrients such as nitrogen and phosphorus, which are vital for plant growth. In addition, the soil's ability to retain water is another important indicator of soil health, as it directly affects the resistance of plants to drought conditions. Monitoring these parameters will provide data on the effectiveness of bioaugmentation techniques and their long-term sustainability [11-14].

Results

Table 1: Soil analysis at the beginning of the experiment: initial state

Test description	Value	U.M.	U	LQ	Method
pH	6,9	pH unit			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met III.1
Assimilable phosphorus	451	mg/Kg di P ₂ O ₅			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XV.3
Exchangeable Potassium *	393	mg/Kg			MEP-S-05 rev. 0 del 22/04/2013
Calcium carbonate	32	g/Kg di CaCO ₃			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met V.2
Total limestone*	76	g/Kg di CaCO ₃			D.M. 13/09/99 SO n° 185 GU n° 248 21/10/1999 Met. V.1
Organic carbon Organic matter	138 238	g/Kg g/Kg			DM 13/09/1999 SO n°185 GU n° 248 21/10/1999 Met.VII 3 DM25/03/2002GU n°84 10/04/2002
Cation exchange capacity*	9,5	meq/100g			DM 13/09/1999 SO n°185 GU n°248 21/10/1999 Met XIII.2 DM 25/03/2002 GU n° 84 10/04/2002
Total nitrogen	10,7	g/Kg	± 0.7		DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIV.2 + XIV.3 + DM 25/03/2002 GU n 84 10/04/2002

LQ: Limit of Quantification – UM: Unit of Measurement – U: Uncertainty

Table 2: Soil analysis at the end of the experiment: Control not treated with CO₂Fixator

Test description	Value	U.M.	U	LQ	Method
pH	7,1	pH unit			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met III.1
Assimilable phosphorus	808	mg/Kg di P ₂ O ₅		5	DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XV.3
Exchangeable potassium *	491	mg/Kg		40	MEP-S-05 rev. 0 del 22/04/2013
Calcium carbonate	39	g/Kg di CaCO ₃			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met V.2
Total nitrogen	83	g/Kg di CaCO ₃			D.M. 13/09/99 SO n° 185 GU n° 248 21/10/1999 Met. V.1
Organic carbon Organic matter	122,8 222,2	g/Kg g/Kg			DM 13/09/1999 SO n°185 GU n° 248 21/10/1999 Met.VII 3 DM25/03/2002GU n°84 10/04/2002
Cation exchange capacity	8,4	meq/100g			DM 13/09/1999 SO n°185 GU n°248 21/10/1999 Met XIII.2 DM 25/03/2002 GU n° 84 10/04/2002
Total nitrogen	11,8	g/Kg	± 0.7		DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIV.2 + XIV.3 + DM 25/03/2002 GU n 84 10/04/2002

LQ: Limit of Quantification – UM: Unit of Measurement – U: Uncertainty

Table 3: Soil analysis at the end of the experiment: Sample treated with CO₂Fixator

Test description	Value	U.M.	U	LQ	Method
pH	7,7	pH unit			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met III.1
Assimilable phosphorus	783	mg/Kg di P ₂ O ₅		5	DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XV.3
Exchangeable potassium *	114	mg/Kg		40	MEP-S-05 rev. 0 del 22/04/2013
Calcium carbonate	44	g/Kg di CaCO ₃			DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met V.2
Total limestone*	111	g/Kg di CaCO ₃			D.M. 13/09/99 SO n° 185 GU n° 248 21/10/1999 Met. V.1
Organic carbon, Organic matter	140.6, 244.4	g/Kg			DM 13/09/1999 SO n°185 GU n° 248 21/10/1999 Met.VII 3
Cation exchange capacity*	9,9	meq/100 g			DM 13/09/1999 SO n°185 GU n°248 21/10/1999 Met XIII.2
Total nitrogen	12,2	g/Kg	± 0.7		DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIV.2 + XIV.3

Table 4: Emissions detected in the agricultural field (ppm):

Average measurements	Control Not treated with CO ₂ Fixator	Sample1 treated with CO ₂ Fixator	Sample2 treated with CO ₂ Fixator	Sample3 treated with CO ₂ Fixator
1	545,65	31,83	70,47	20,13
2	329,86	28,15	102,53	17,49
3	651,54	31,09	105,38	31,09
4	1682,4	29,96	113,19	83,51

5	451,4	37,03	117,21	21,1
6	408,05	29,87	108,78	23,76
7	1230,02	31,55	112,85	70,7
8	1444,9	31,36	112,18	73,27
9	349,51	29,83	112,68	17,37
10	354,28	30,24	114,22	12,43
11	5892,06	43,81	142,08	215,45
12	860,79	35,42	116,18	28,63
13	630,33	28,83	108,88	24,32
14	2157,55	50,03	134,13	97,23
15	862,17	31,31	114,02	36,58
16	718,34	29,81	114,54	23,81
17	762,38	27,35	115,58	25,82
18	645,06	28,86	109	28,86
19	964,17	30,01	111,3	30,01
20	444,32	28,94	111,21	17,03
21	1142,24	34,32	116,65	39,91
22	703,57	34,63	123,95	26,35
23	293,49	30,22	112,07	10,92
24	605,16	27,37	113,31	17,98
25	337,03	30,38	112,65	14,64
26	1154,07	30,17	111,87	49,7
27	568,28	29,07	113,86	20,49
28	3005,85	32,42	113,95	118,09
29	379,56	27,1	106,12	14,76
30	846,26	29,12	111,92	24,65
31	922,94	31,12	123,91	36,46
32	934,88	26,07	107,91	35,88
33	343,86	24,9	101,47	12,32
34	238,07	29,89	117,07	12,29
35	2310,83	28,19	116,7	86,4
36	1344,86	31,42	112,36	56,68
37	454,15	29,39	115,09	22,02
38	1023,67	27,98	107,74	38,52
39	5848,64	41,16	128,99	270,31
40	1624,96	37,03	128,19	79,25
41	1034,56	38,6	127,08	44,66
42	747,81	40,51	135,9	38,56
43	434,89	48,96	205,13	23,74
44	489,3	51,19	244	28,1
45	393,37	51,15	236,96	25,1
46	444,13	51,1	243,57	28,05
47	797,01	51,35	244,77	44,66
48	831,15	49,08	238,04	44,69
49	6424	61,31	251,88	375,71
50	2453,33	56,26	238,29	145,69
51	900,86	49,05	224,52	56,17
52	479,76	46,85	211,85	31,44
53	639,28	43,19	202,28	37,23

54	541,78	44,93	194,62	29,95
55	541,03	44,87	188,5	29,91

Table 5: Emissions detected in the laboratory (ppm)

Average measurements	Control Not treated with CO2Fixator	Sample1 treated with CO2Fixator	Sample2 treated with CO2Fixator	Sample3 treated with CO2Fixator
1	567,15	23,74	34,07	24,77
2	597,99	23,74	34,07	28,02
3	537,56	23,74	34,07	22,75
4	490,91	23,74	34,07	20,85
5	518,51	22,75	34,07	22,75
6	499,98	22,75	34,07	21,79
7	455,88	22,75	34,07	19,94
8	406,96	22,75	32,8	18,21
9	430,89	22,75	34,07	19,06
10	391,58	22,75	34,07	16,58
11	1418,31	21,79	32,8	67,54
12	455,88	22,75	32,8	22,75
13	414,82	21,79	32,8	20,85
14	384,05	21,79	32,8	19,94
15	255,22	21,79	32,8	12,95
16	490,91	20,85	32,8	19,94
17	384,05	20,85	32,8	17,38
18	223,82	20,85	32,8	11,05
19	686,63	21,79	31,55	44
20	1862,48	21,79	32,8	83,34
21	1418,31	21,79	32,8	67,54
22	384,05	30,34	39,53	19,06
23	327,74	26,9	36,73	16,58
24	308,32	26,9	36,73	16,58
25	289,78	25,82	35,39	15,8
26	233,95	23,74	34,07	12,95
27	260,75	24,77	35,39	12,29
28	1259,32	22,75	34,07	59,54
29	587,57	29,16	45,57	28,02
30	406,96	29,16	47,17	19,06
31	430,89	28,02	47,17	22,75
32	327,74	26,9	36,73	16,58
33	641,12	52,22	40,98	31,55
34	228,84	30,34	47,17	12,29
35	218,88	29,16	47,17	11,05
36	209,25	29,16	47,17	10,47
37	295,87	28,02	47,17	17,38
38	1081,16	28,02	45,57	45,57
39	675,02	29,16	47,17	28,02
40	557,15	29,16	45,57	24,77
41	490,91	29,16	45,57	22,75
42	447,43	28,02	45,57	19,94
43	473,14	28,02	45,57	22,75
44	1185,42	28,02	45,57	53,99

45	341,19	29,16	47,17	16,58
46	289,78	28,02	47,17	14,33
47	283,79	26,9	45,57	14,33
48	327,74	26,9	45,57	20,85
49	289,78	26,9	47,17	13,63
50	430,89	26,9	47,17	21,79
51	1639,69	26,9	45,57	47,17
52	308,32	26,9	40,98	19,06
53	348,07	25,82	40,98	19,06
54	177,97	25,82	42,47	8,83
55	266,37	29,16	44	15,05

Table 6: Gains/loss percentages

Value	Control	Sample
Phosphorus	79%	74%
Potassium	24%	-71%
Calcium carbonate	22%	+37,5%
Total limestone	9%	46%
Organic carbon	-6,7%	2%
Organic matter	-6,7%	+2,5%
Cation exchange capacity	-12%	4%

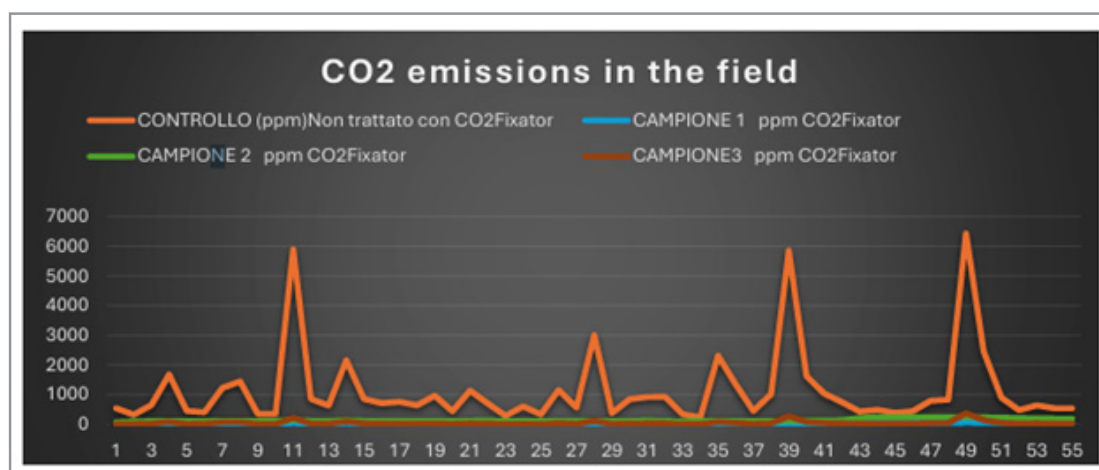


Figure 1: Emissions measured in the field (average over 24000 measurements) y: ppm; x: average measurements

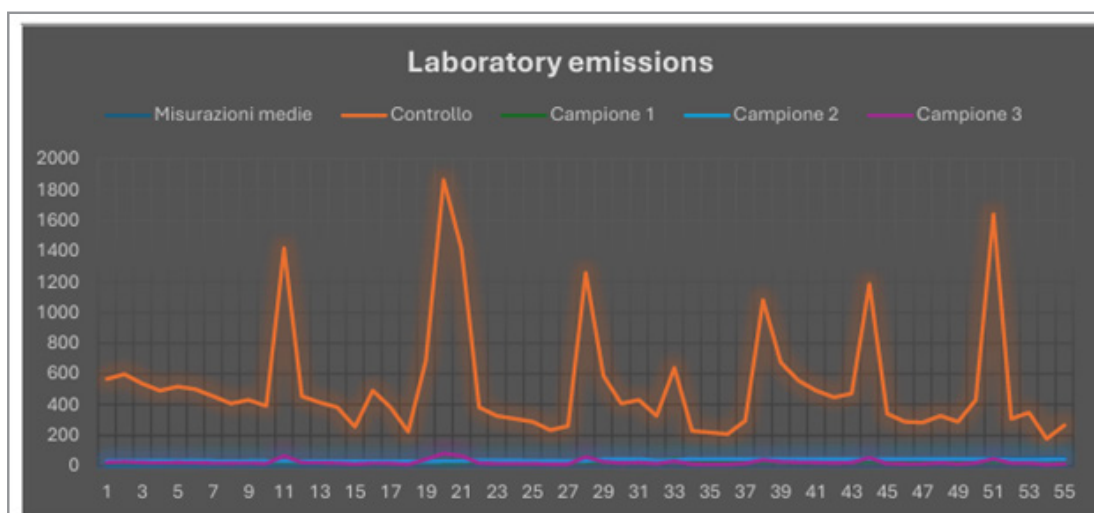


Figure 2: Emissions measured in the laboratory (average over 24000 measurements) y: ppm; x: average measurements



Figure 3: Gains/losses of control and samples compared to the initial state

Discussion

To facilitate the comparison between the results of the initial sample and the control sample, it is highlighted how the organic substance is metabolized by bacteria normally present in the soil, resulting in CO₂ as a catabolite [15-17]. The element of greatest interest in this study is organic carbon, a component of the soil organic substance consisting essentially of carbon present in organic compounds, including carbon in molecules such as carbohydrates, proteins, lipids and nucleic acids. These residues undergo processes of decomposition, fermentation and transformation operated by living organisms present in the soil [18]. The decrease in the organic component in the control sample clearly suggests that this substance was metabolized by the bacterial component of the soil with the production of CO₂ as a catabolite. On the contrary, both the field sample and the laboratory sample treated with the CO₂Fixator show an increase in the organic carbon component. The data obtained with the specific sensor for CO₂ "SCD30" clearly indicate that the concentration of CO₂ (expressed in ppm, parts per million) in the control sample (orange) is higher than that of the treated samples. It is observed that, while the control sample emits CO₂ discontinuously and in high quantities compared to the treated samples that emit CO₂ continuously and in significantly lower quantities. However, the detection of CO₂ by sensor can only provide qualitative data, since the gas tends to accumulate under the containers. In the graph of gains/losses of substances expressed as a percentage compared to the initial state, the control is in yellow, the sample is in orange. Organic carbon increases significantly in the samples treated with the CO₂Fixator [19]. The development of new seedlings was observed in the control and in the sample, in the control the development of new seedlings did not affect the compensation of CO₂ emissions from the soil, indicating that the rate of decomposition of the organic component of the soil exceeds the activity of CO₂ fixation by the new organic substance. On the contrary, in the treated sample the development of new seedlings adds and fixes more CO₂. The laboratory data provide us with clear indications of the effects of the CO₂Fixator also in other aspects. An increase in the cation exchange capacity is recorded in the treated sample, which allows the retention of essential nutrients that can be released to the plants when necessary [20-24]. It is also an indication of a greater soil structure with water retention capacity, while in the untreated sample a reduction of the same is evident compared to the sam-

ple of the initial state. The same can be said of the total limestone component that has influenced the pH, alkalizing the soil, and of total nitrogen. The decomposition process brought phosphorus to the control and treated samples, it is noted that potassium in the control has slightly increased while in the treated sample it has drastically decreased, this is due to the development of new seedlings that are qualitatively superior to the seedlings grown in the control sample [25, 26].

Conclusions

CO₂Fixator is an innovative product composed of a mix of bacteria and the fungus *Trichoderma viride*, designed to fix CO₂ to the soil and improve the quality of the soil. The technology behind CO₂Fixator exploits the synergistic effect between bacteria and the fungus *Trichoderma viride* [27-30]. This synergistic effect allows CO₂ to be fixed to the soil, contributing to the reconstruction of the soil. Each bacterium in CO₂Fixator has specific metabolic capabilities, attacking only the substrate that provides nutrients and releasing molecules that have antibiotic use into the surrounding environment, preventing other bacteria from replicating and spreading, promoting plant growth. Although *Bacillus subtilis* and *Bacillus licheniformis* produce CO₂ as a by-product of their metabolism, the amount of carbon dioxide emitted is significantly lower than that produced by bacteria normally present in the soil. The presence of other microorganisms in the CO₂Fixator ensures that the substrate is digested and metabolized into simple, ready-to-use components, promoting mutual nourishment. The other bacteria in the CO₂Fixator produce CO₂ only as a secondary by-product and only under particular physical-chemical conditions [31-35]. The synergistic action of the CO₂Fixator promotes soil reconstruction by preventing erosion, water retention prevents soil leaching and the loss of mineral salts and organic matrix, an increase in soil volume corresponds to an increase in soil fertility. *Trichoderma viride* regulates the vegetative processes of the plant, harmonizing the absorption of nutrients after the composting action of the bacterial mix. The effectiveness of the preparation demonstrates that its use could not be limited to the digestion of soil organic matter, but could also be extended to other types of organic substances such as manure or sewage sludge. Quantity and quality could be the subject of further studies. The greater presence of organic matter in the treated samples is due to a series of factors that, together, constitute a synergistic pool. This pool not only

reduces CO₂ emissions, but also favors the development of additional plants that absorb other CO₂, making the soil an ideal Carbon Sink. The study has provided further elements relating to the improvement of soil quality, making agricultural practice more sustainable. In addition to the increase in the organic and inorganic component, with a consequent increase in soil volume, an improvement in water retention capacity is also observed [36-40]. The bioavailability of nitrogen and phosphorus, together with the soil's ability to provide water and the action of *Trichoderma viride*, which favors rooting, allow obtaining quality soil without the addition of additional substances. However, it is important to note the impoverishment of potassium that should be added to the mix to obtain the maximum synergistic effect and support bioaugmentation. The CO₂Fixator transforms agricultural soil into a Carbon Sink of choice, simultaneously improving its quality [41-43].

The amount of CO₂ fixed in the soil equivalent to the amount of organic carbon is given by the ratio between the molecular mass of CO₂ and the molecular mass of Carbon

1 tonne of fixed carbon is equivalent to 3.66 tonnes of atmospheric CO₂.

For example:

- the residual biomass of corn on a hectare varies between 5 and 15 tonnes of dry matter, between 18.3 and 54.9 tonnes of CO₂ would be fixed;
- the residual biomass of wheat on a hectare varies between 3 and 8 tonnes of dry matter, between 10.98 and 29.28 tonnes of CO₂ would be fixed;
- the residual rice biomass on one hectare varies between 3 and 7 tons of dry matter, between 10.98 and 25.62 tons of CO₂ would be fixed;
- The carbon content in one ton of manure varies between 150 and 300Kg, between 0.549 – 1.098 tons of CO₂ would be fixed;
- The carbon content in one ton of dry matter derived from sewage sludge varies between 250 – 350Kg, between 0.915 – 1.281Kg would be fixed [44].

In conclusion, compared to other techniques and systems of CO₂ capture, such as reforestation, algae cultivation, air filtration or conversion of CO₂ into fuel, the CO₂Fixator can be applied anywhere there is land, whether agricultural or not, including organic waste, sewage sludge and organic fertilizer. In the specific case of agricultural soil, bacterial digestion involves the bioaugmentation of soil components, resulting in soil regeneration and making it progressively more fertile treatment after treatment. The total agricultural area (SAT) in Italy amounts to approximately 26.2 million hectares, including all lands intended for crops, pastures, permanent grasslands and other agricultural surfaces [45-47]. Assuming an average fixation of 15 tonnes of CO₂ per hectare, a total of 393 million tonnes of CO₂ are fixed each year. In 2021, total greenhouse gas emissions in Italy, expressed in CO₂ equivalent, were approximately 418 million tonnes. Widespread adoption of CO₂Fixator could help offset 94% of Italy's total emissions.

References

1. Mahapatra, S., & Yadav, R. (2022). *Bacillus subtilis* impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. *Journal of Applied Microbiology*. <https://doi.org/10.1093/jambio/ixac038>

2. Todorova, S., & Kozhuharova, L. (2010). Characteristics and antimicrobial activity of *Bacillus subtilis* strains isolated from soil. *World Journal of Microbiology and Biotechnology*, 26(7), 1207–1216. <https://doi.org/10.1007/s11274-009-0290-1>
3. Wipat, A., & Harwood, C. R. (1999). The *Bacillus subtilis* genome sequence: The molecular blueprint of a soil bacterium. *FEMS Microbiology Ecology*, 28(1), 1–9. <https://doi.org/10.1111/j.1574-6941.1999.tb00555.x>
4. Sun, B., Gu, L., Bao, L., Zhang, S., Wei, Y., & Bai, Z. (2020). Application of biofertilizer containing *Bacillus subtilis* reduced the nitrogen loss in agricultural soil. *Soil Biology and Biochemistry*, 148, 107912. <https://doi.org/10.1016/j.soilbio.2020.107912>
5. Ng, C. W. W., Yan, W. H., Tsim, K. W. K., So, P. S., & Xia, Y. T. (2022). Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* as the soil amendment. *Heliyon*, 8(9), e10613. <https://doi.org/10.1016/j.heliyon.2022.e10613>
6. Hong, Z., Rong, X., Cai, P., Dai, K., Liang, W., & Huang, Q. (2012). Initial adhesion of *Bacillus subtilis* on soil minerals as related to their surface properties. *European Journal of Soil Science*, 63(4), 457–465. <https://doi.org/10.1111/j.1365-2389.2012.01449.x>
7. Ma, W., Peng, D., Walker, S. L., Cao, B., & Gao, C. H. (2017). *Bacillus subtilis* biofilm development in the presence of soil clay minerals and iron oxides. *npj Biofilms and Microbiomes*, 3, 18. <https://doi.org/10.1038/s41522-017-0026-9>
8. Sun, B., Bai, Z., Bao, L., Xue, L., Zhang, S., Wei, Y., & Shen, J. (2020). *Bacillus subtilis* biofertilizer mitigating agricultural ammonia emission and shifting soil nitrogen cycling microbiomes. *Environment International*, 140, 105766. <https://doi.org/10.1016/j.envint.2020.105766>
9. Chen, W., Wu, Z., Liu, C., Zhang, Z., & Liu, X. (2023). Biochar combined with *Bacillus subtilis* SL-44 as an eco-friendly strategy to improve soil fertility, reduce Fusarium wilt, and promote radish growth. *Ecotoxicology and Environmental Safety*, 248, 114382. <https://doi.org/10.1016/j.ecoenv.2023.114382>
10. Lowe, M. A., Mathes, F., Loke, M. H., McGrath, G., & Murphy, D. V. (2019). *Bacillus subtilis* and surfactant amendments for the breakdown of soil water repellency in a sandy soil. *Geoderma*, 338, 124–133. <https://doi.org/10.1016/j.geoderma.2018.11.042>
11. Ghaly, A. E., Arab, F., & Mahmoud, N. S. (2007). Production of levan by *Bacillus licheniformis* for use as a soil sealant in earthen manure storage structures. *American Journal of Biochemistry and Biotechnology*, 3(2), 47–54. <https://doi.org/10.3844/ajbbsp.2007.47.54>
12. Vahabi, A., Ramezani-pour, A. A., & Yousefi, A. A. (2015). Calcium carbonate precipitation by strain *Bacillus licheniformis* AK01, newly isolated from loamy soil: A promising alternative for sealing cement-based materials. *Journal of Basic Microbiology*, 55(12), 1474–1481. <https://doi.org/10.1002/jobm.201400906>
13. Ni, S., Wu, Y., Zhu, N., Leng, F., & Wang, Y. (2024). *Bacillus licheniformis* YB06: A rhizosphere-genome-wide analysis and plant growth-promoting analysis of a plant growth-promoting rhizobacterium. *Microorganisms*, 12(2), 334. <https://doi.org/10.3390/microorganisms12020334>

14. Gulhane, P. A., Gomashe, A. V., & Lade, S. (2014). *Bacillus licheniformis* NCIM 2536. International Journal of Current Microbiology and Applied Sciences, 3(6), 205–211. <http://www.ijcmas.com/vol-3-6/P.%20A.%20Gulhane,%20et%20al.pdf>
15. Shariat, P., Mitchell, W. J., Boyd, A., & Priest, F. G. (1995). Anaerobic metabolism in *Bacillus licheniformis* NCIB 6346. Microbiology, 141(10), 2573–2580. <https://doi.org/10.1099/13500872-141-10-2573>
16. Chang, Y., Zhou, K., Yang, T., Zhao, X., Li, R., Li, J., & Xu, S. (2023). *Bacillus licheniformis* inoculation promoted humification process for kitchen waste composting: Organic components transformation and bacterial metabolic pathways. Environmental Research, 216, 114809. <https://doi.org/10.1016/j.envres.2022.114809>
17. Su, J., Zhou, K., Chen, W., Xu, S., Feng, Z., & Chang, Y. (2024). Enhanced organic degradation and microbial community cooperation by inoculating *Bacillus licheniformis* in low temperature composting. Journal of Environmental Management, 351, 119943. <https://doi.org/10.1016/j.jenvman.2024.119943>
18. Clements, L. D., Miller, B. S., & Streips, U. N. (2002). Comparative growth analysis of the facultative anaerobes *Bacillus subtilis*, *Bacillus licheniformis*, and *Escherichia coli*. Systematic and Applied Microbiology, 25(2), 284–286. <https://doi.org/10.1078/0723-2020-00110>
19. Degrange, V., Lensi, R., & Bardin, R. (1997). Activity, size and structure of a *Nitrobacter* community as affected by organic carbon and nitrite in sterile soil. FEMS Microbiology Ecology, 24(2), 173–180. <https://doi.org/10.1111/j.1574-6941.1997.tb00392.x>
20. Sorrenti, G., Buriani, G., Gaggia, F., Baffoni, L., & Spinelli, F. (2017). Soil CO₂ emission partitioning, bacterial community profile and gene expression of *Nitrosomonas* spp. and *Nitrobacter* spp. of a sandy soil amended with biochar and digestate. Applied Soil Ecology, 119, 242–251. <https://doi.org/10.1016/j.apsoil.2017.07.015>
21. Wang, X. F., Li, J., Li, G., Zhang, G. L., Wang, Z. W., & Zhi, Y. C. (2022). Biochar application affects *Nitrobacter* rather than *Nitrospira* in plastic greenhouse vegetable soil. Applied Soil Ecology, 177, 104533. <https://doi.org/10.1016/j.apsoil.2022.104533>
22. Quastel, J. H., & Scholefield, P. G. (1951). Biochemistry of nitrification in soil. Bacteriological Reviews, 15(1), 1–53. <https://doi.org/10.1128/br.15.1.1-53.1951>
23. Li, Y., Chapman, S. J., Nicol, G. W., & Yao, H. (2018). Nitrification and nitrifiers in acidic soils. Soil Biology and Biochemistry, 116, 290–301. <https://doi.org/10.1016/j.soilbio.2017.10.017>
24. Liu, W., Zhao, L., Chen, Y., Shen, Y., & Luo, Z. (2023). Soil properties and silage quality in response to oat and pea seeding ratios and harvest stage on the Qinghai-Tibetan Plateau. Frontiers in Sustainable Food Systems, 7, 1186501. <https://doi.org/10.3389/fsufs.2023.1186501>
25. Limanska, N., Ivanytsia, T., Basiul, O., & Krylova, K. (2013). Effect of *Lactobacillus plantarum* on germination and growth of tomato seedlings. Acta Physiologiae Plantarum, 35, 3147–3150. <https://doi.org/10.1007/s11738-013-1344-6>
26. Khan, A. N., Hassan, M. N., Keyani, R., & Amir, H. Z. (2024). Potential of *Lactobacillus agilis*, *Lactobacillus plantarum*, and *Lactobacillus acidophilus* to enhance wheat growth under drought and heat stress. Journal of King Saud University - Science, 36(2), 103739. <https://doi.org/10.1016/j.jksus.2023.103739>
27. Zhu, G., Li, Y., Cheng, D., Chen, R., Wang, Y., & Tu, Q. (2024). Effects of Distiller's Grains Biochar and *Lactobacillus plantarum* on the remediation of Cd-Pb-Zn-contaminated soil and growth of sorghum-sudangrass. Microorganisms, 12(2), 387. <https://doi.org/10.3390/microorganisms12020387>
28. Su, J., Xue, Y., Zhang, K., Liu, Z., Lv, J., Yang, Q., Li, Z., & Liu, C. (2024). The effects of *Lactobacillus plantarum*, *Bacillus subtilis*, a lignocellulolytic enzyme system, and their combination on the fermentation profiles and chemical composition of silage. Animals, 14(4), 568. <https://doi.org/10.3390/ani14040568>
29. Claus, G., & Kutzner, H. J. (1985). Physiology and kinetics of autotrophic denitrification by *Thiobacillus denitrificans*. Applied Microbiology and Biotechnology, 22(4), 283–288. <https://doi.org/10.1007/BF00252738>
30. Sublette, K. L., & Sylvester, N. D. (1987). Oxidation of hydrogen sulfide by *Thiobacillus denitrificans*: Desulfurization of natural gas. Biotechnology and Bioengineering, 29(2), 249–257. <https://doi.org/10.1002/bit.260290211>
31. Baalsrud, K., & Baalsrud, K. S. (1954). Studies on *Thiobacillus denitrificans*. Archiv für Mikrobiologie, 21, 344–362. <https://doi.org/10.1007/BF00410169>
32. Blažková, Z. (n.d.). Nitrates removal from wastewater using bacteria *Thiobacillus denitrificans* [Master's thesis, University of Pardubice]. <https://dk.upce.cz/handle/10195/68942>
33. Lucheta, A. R., & Lambais, M. R. (2012). Sulfur in agriculture. Revista Brasileira de Ciência do Solo, 36(5), 1369–1379. <https://doi.org/10.1590/S0100-06832012000500001>
34. Al-Amri, M., Souissi, Y., Menezes-Blackburn, D., & Wichern, F. (2024). Bacterial communities associated with the oxidation of elemental sulfur in calcareous soils of Oman. Journal of Soil Science and Plant Nutrition, 24, 165–179. <https://doi.org/10.1007/s42729-024-01513-5>
35. Waksman, S. A. (1922). Microorganisms concerned in the oxidation of sulfur in the soil I. Introductory. Journal of Bacteriology, 7(2), 239–256. <https://doi.org/10.1128/jb.7.2.239-256.1922>
36. Chen, G., Zhu, H., & Zhang, Y. (2003). Soil microbial activities and carbon and nitrogen fixation. Research in Microbiology, 154(5), 393–398. [https://doi.org/10.1016/S0923-2508\(03\)00086-8](https://doi.org/10.1016/S0923-2508(03)00086-8)
37. Wake, L. V. (1972). Isolation and properties of methane utilizing bacteria (Master's thesis). University of New South Wales. <https://unsworks.unsw.edu.au/entities/publication/2c4cf9c1-2975-4a87-998a-f9aa24df44df>
38. Soil Science Society of America. (n.d.). Soil Science Society of America Journal. Wiley. <https://access.onlinelibrary.wiley.com/journal/14353043>
39. Soil and Water Conservation Society. (n.d.). Journal of Soil and Water Conservation. Soil and Water Conservation Society. <https://www.jswnonline.org/>
40. Elsevier. (n.d.). Soil Biology and Biochemistry. Elsevier. <https://www.journals.elsevier.com/soil-biology-and-biochemistry/>
41. Elsevier. (n.d.). Geoderma. Elsevier. <https://www.journals.elsevier.com/geoderma/>

-
42. Elsevier. (n.d.). Agriculture, Ecosystems and Environment. Elsevier. <https://www.journals.elsevier.com/agriculture-ecosystems-and-environment/>
 43. Springer. (n.d.). Plant and Soil. Springer Nature. <https://www.springer.com/journal/11104>
 44. Elsevier. (n.d.). Applied Soil Ecology. Elsevier. <https://www.journals.elsevier.com/applied-soil-ecology/>
 45. Elsevier. (n.d.). Soil and Tillage Research. Elsevier. <https://www.journals.elsevier.com/soil-and-tillage-research/>
 46. British Society of Soil Science. (n.d.). European Journal of Soil Science. Wiley. <https://bsssjournals.onlinelibrary.wiley.com/journal/13652389>
 47. Cambridge University Press. (n.d.). The Journal of Agricultural Science. Cambridge University Press. <https://www.cambridge.org/core/journals/journal-of-agricultural-science>