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# Agricultural Soil as a Carbon Sink

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***Abstract***

***Aims:*** *CO2Fixator is the commercial name given to a microbiological preparation consisting of a mixture of bacterial strains, consisting of Bacillus subtilis, Bacillus licheniformis, Nitrobacter, Lactobacillus plantarum, Thiobacillus denitrificans, and Methilococcus capsulatus; and a fungal strain Trichoderma viride. The bacterial strains metabolize organic soil substrates, fixing carbon in the soil and reducing carbon emissions in the form of gaseous CO2. Additionally, Trichoderma viride promotes the development of new plants. The aim of this study is to demonstrate that a bacterial and fungal mixture can be used in sustainable agricultural practices to reduce the carbon footprint of agriculture: carbon and nutrients such as nitrogen and phosphorus are fixed in the soil, greenhouse gas emissions are reduced by limiting carbon volatility, and the water retention capacity and cation exchange capacity of the soil increase.*

***Methods:*** *This method involves the use of CO2 sensors and laboratory analyses to quantify substances present in the soil and verify that CO2 fixation has occurred.*

***Results:*** *This study provides evidence to demonstrate that CO2Fixator increases the quantity of soil carbon and soil quality. Conclusions: The mixture transforms agricultural soil into a carbon sink while improving soil quality.*

***Discussion:*** *Organic substances are metabolized by bacteria normally present in the soil, with CO2 as a catabolite. The element of greatest interest in this study is organic carbon, a component of soil that essentially consists of carbon present in organic compounds, which includes carbon in molecules such as carbohydrates, proteins, lipids and nucleic acids.*

**Keywords:** Soil Carbon, Climate Change Mitigation, Soil Organic Matter, Greenhouse Gases, Carbon Cycle, Soil Health, Sustainable Land Management, Environmental Policy

## 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 the fertility of soil and its ability to produce abundant crops but also helps reduce greenhouse gas emissions by improving the structure of soil 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. These practices regenerate the soil and also create an environment that is 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 more ecological and productive agriculture practices that respect the natural balance and contribute to the fight against global warming, which regularly causes extreme weather events with enormous economic costs each year. 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, ploughing residues into the soil, and 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.

"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 crop residues so that the carbon captured by plants is not released back into the atmosphere, as well as 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. 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 of these methods is to develop agricultural systems that avoid releasing CO2 and 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. CO2Fixator, the subject of this study, presents an approach that prevents 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, including the significant opportunities presented by the use of agricultural waste as fertilizer and the generation of carbon credits. 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. For example, a farmer who switches from traditional ploughing to strip tilling or no-till practices, adopts burial or injection systems of slurry and digestates, and 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 specified 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. 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 generated carbon credits and participate in a national voluntary market, which is in line with the provisions related to the National Registry of Agro-Forestry Carbon Sink. 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 formulation described incorporates a combination of bacterial and fungal strains, each with specific characteristics that contribute to soil health, plant growth and environmental sustainability. Below, I summarize the main information:

1. Bacillus subtilis Dr. Jekyll et al. (2022) S. Todorova et al. (2010) B. Sun et al. (2020); 2. Bacillus licheniformis Y. Chang et al. (2023)J. Su et al. (2024); 3. Nitrobacter V. Degrange et al. (1997)J.H. Quastel et al. (1951); 4. Lactobacillus plantarum J. Su et al. (2024); 5. Thiobacillus denitrificans: G. Claus (1985); 6. Methilococcus capsulatus L.V. Wake et al. (1972)

- **Fungus:** Trichoderma viride Lourdes Macías-Rodríguez et al. (2016).

The concentration of Bacillus subtilis was 5×109 CFU/g, while for the concentration of the other bacterial strains and the fungus Trichoderma viride was 1×109 CFU/g.

The formulation complied with the following percentage composition expressed in grams per litre (g/L), divided into proportional parts:

- **Bacillus subtilis:** 15 parts (15%), corresponding to 0.15 g/L
- **Bacillus licheniformis:** 20 parts (20%), corresponding to 0.20 g/L
- **Nitrobacter:** 10 parts (10%), corresponding to 0.10 g/L
- **Lactobacillus plantarum:** 15 parts (15%), corresponding to 0.15 g/L
- **Thiobacillus denitrificans:** 15 parts (15%), corresponding to 0.15 g/L
- **Methilococcus capsulatus:** 15 parts (15%), corresponding to 0.15 g/L
- **Trichoderma viride:** 10 parts (10%), corresponding to 0.10 g/L

All components were carefully weighed and mixed to obtain a uniform and homogeneous distribution in microbiological powder, ensuring it was ready for use in experiments. A total of 3.3 L of solution was used for the preparation. Using closed chambers, a controlled environment was created to monitor emissions. These chambers, made from inverted plastic containers, were sealed to the ground to capture gases emitted by buried and decaying crop residues. Special holes were made in the chambers to allow gas exchange with the external environment. Inside the chambers, "SCD30" model CO2 gas sensors continuously monitored the carbon dioxide concentrations, providing detailed information on the dynamics of emissions and the effectiveness of the CO2Fixator device in reducing environmental impact. The sensors were calibrated to detect gas concentrations, expressed in parts per million (ppm), starting from a minimum of 10 ppm without upper limits, ensuring maximum precision and

reliability of the collected data. A total of 24,000 measurements were collected, and the average values of were calculated every 437 measurements, which are reported in Tables 4 (emissions detected in the agricultural field, Fig. 1) and 5 (emissions detected in the laboratory, Fig. 2). The experiment was conducted in duplicate, both in the agricultural field and in the laboratory. The values of the soil used before the start of the experiment are reported in Table 1. In the laboratory, the tests were carried out while maintaining a constant temperature of 20°C and humidity of 50%. For both conditions, the soil was prepared with a uniform inorganic matter to dry matter composition of 5:1. This ratio corresponds to approximately 27,778 tons of dry organic matter per hectare and approximately 100 g for each container in the laboratory. The analyses allowed us to determine the amount of organic matter present and to verify whether the introduction of CO2Fixator was able to improve the availability of essential nutrients that are crucial for plant growth, such as nitrogen and phosphorus. Furthermore, the capacity of the soil to retain water was considered an important indicator of soil health, directly influencing the ability of plants to withstand drought. The results obtained after three months of experimentation are presented in Tables 2 (control) and 3 (treated sample). Table 6, supported by Figure 3, reports the percentage values related to the gains and losses of soil components. Both the control (500 g) and the treated samples (mixed to obtain a total of 500 g) were subjected to analysis.

## Results

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

| Test description | Value | U.M. | U | LQ | Method |
|---|---|---|---|---|---|
| Soil texture | 875 | g/Kg | | | DM 13/09/1999 SO n° 185 GU n° |
| Sand | 75 | g/Kg | | | 248 21/10/1999 Met II.6 DM |
| Silt | 50 | g/Kg | | | 25/03/2002 GU n° 84 10/04/2002 |
| Clay | 6,9 | pH unit | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met III.1 |
| pH | 451 | mg/Kg di P2O5 | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XV.3 |
| Assimilable phosphorus | 393 | mg/Kg | | | MEP-S-05 rev. 0 del 22/04/2013 |
| Exchangeable potassium | 32 | g/Kg di CaCO3 | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met V.2 |
| Calcium carbonate | 76 | g/Kg di CaCO3 | | | D.M. 13/09/99 SO n° 185 GU n° 248 21/10/1999 Met. V.1 |
| Total limestone | 138 | 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 |
| Organic carbon Organic matter | 238 | g/Kg | | | 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 |
| Cation exchange capacity | 9,5 | meq/100 g | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIV.2 |
| Total nitrogen | 10,7 | g/Kg | ± 0.7 | | + 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 P2O5 | | 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 CaCO3 | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met V.2 |
| Total limestone* | 83 | g/Kg di CaCO3 | | | D.M. 13/09/99 SO n° 185 GU n° 248 21/10/1999 Met. V.1 |
| Organic carbon Organic matter | 122,8<br>222,2 | g/Kg<br>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/100 g | | | 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 |
| Organic carbon Organic matter | 238 | g/Kg | | | 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 |
| Cation exchange capacity | 9,5 | meq/100 g | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met XIV.2 |
| Total nitrogen | 10,7 | g/Kg | ± 0.7 | | + 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 | unità di pH | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met III.1 |
| Assimilable phosphorus | 783 | mg/Kg di P2O5 | | 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 CaCO3 | | | DM 13/09/1999 SO n 185 GU n 248 21/10/1999 Met V.2 |
| Total limestone* | 111 | g/Kg di CaCO3 | | | D.M. 13/09/99 SO n° 185 GU n° 248 21/10/1999 Met. V.1 |
| Organic carbon Organic matter | 140,6 | 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 CO2Fixator | Sample1 treated with CO2Fixator | Sample2 treated with CO2Fixator | Sample3 treated with CO2Fixator |
|---|---|---|---|---|
| 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% |
| Nitrogen | 10% | 14% |

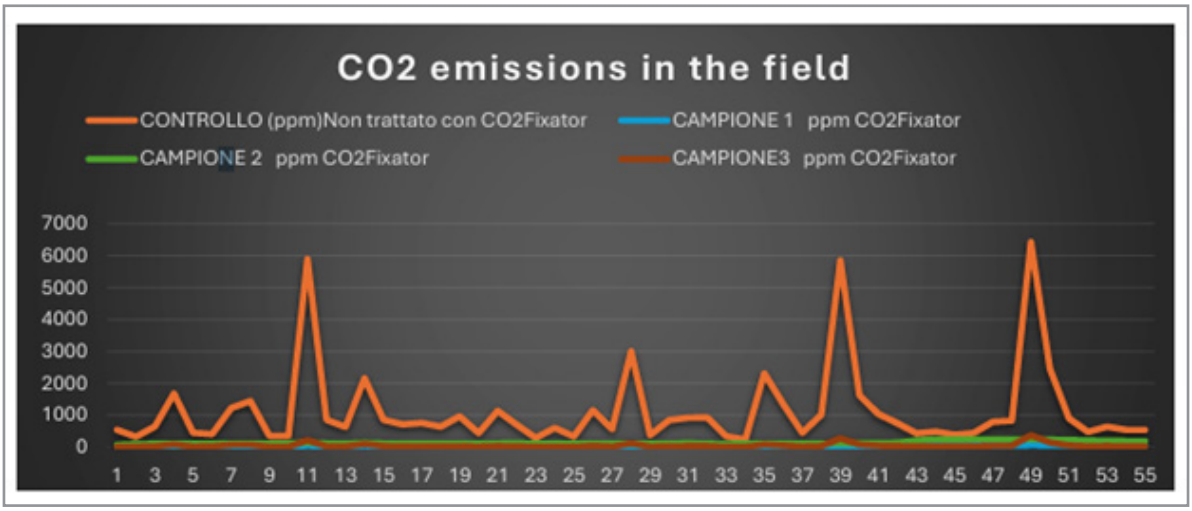


**Figure 1:** Emissions measured in the field (average over 24000 measurements) y: ppm; x: average measurements

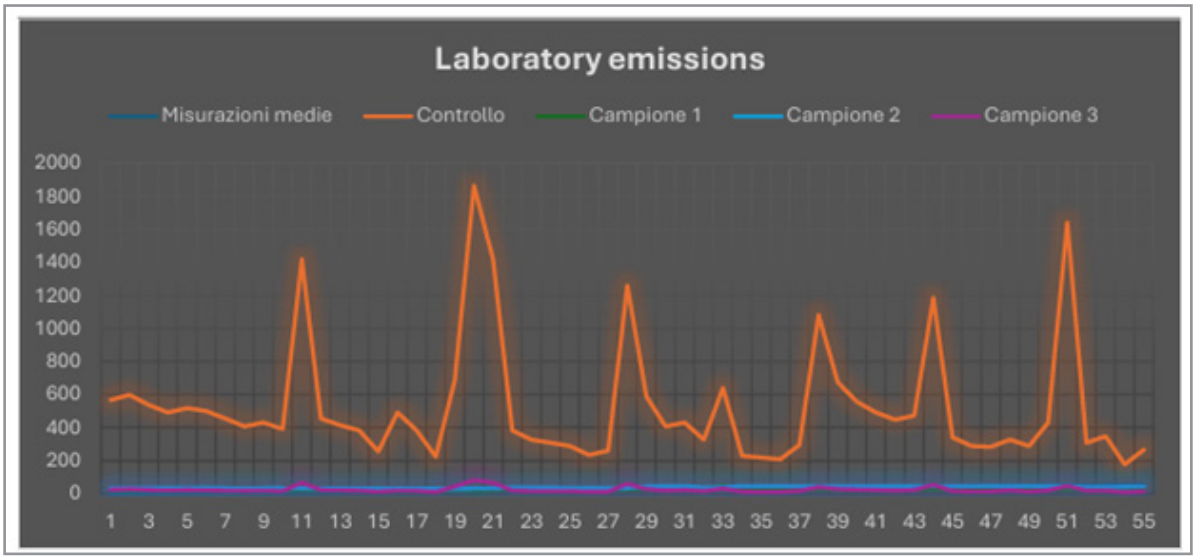


**Figure 2:** Emissions measured in the laboratory (average over 24000 measurements) y: ppm; x: average measurements


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

## Discussion

Organic substances are metabolized by bacteria normally present in the soil, with CO2 as a catabolite. The element of greatest interest in this study is organic carbon, a component of soil that essentially consists of carbon present in organic compounds, which includes carbon in molecules such as carbohydrates, proteins, lipids and nucleic acids. These residues undergo decomposition, fermentation and transformation via living organisms present in the soil. The decrease in the organic component in the control sample clearly suggests that organic carbon was metabolized by the bacterial component of the soil with the production of CO2 as a catabolite. In contrast, both the field sample and the laboratory sample treated with CO2Fixator presented an increase in the organic carbon component of soil. The data obtained from the SCD30 sensor for CO2 clearly indicated that the concentration of CO2 (expressed in ppm, parts per million) in the control sample was greater than that in the treated samples. However, the sensors to detect CO2 can provide only qualitative data, since the gas tends to accumulate under the containers. In Fig. 1, the gains/losses of substances are expressed as a percentage compared with the initial state; the control is in blue, and the sample is in orange. The organic carbon content increased significantly in the samples treated with CO2Fixator. The development of new seedlings was observed in both the control and treated samples. In the control, the development of new seedlings did not affect CO2 emissions from the soil, indicating that the rate of decomposition of organic matter in the soil exceeded the rate of CO2 fixation in the form of new organic substance. In contrast, in treated samples, the development of new seedlings resulted in more CO2 being added and fixed in the soil. The laboratory data provide us with clear indications of the effects of CO2Fixator in addition to other information. An increase in the cation exchange capacity was observed in treated samples, which allows for the retention of essential nutrients that can be released to plants as necessary. This is also an indication of improved soil structure and greater water retention capacity, whereas in the untreated sample, a reduction in the water retention capacity was observed compared with that of the sample in the initial state. Similarly, the total limestone content influences soil pH, alkalizes the soil, and increases the total nitrogen content. The decomposition process resulted in an increase in phosphorus in the control and treated samples. The potassium content in the control slightly increased while that in the treated sample drastically decreased, which occurred due to the development of new seedlings that were qualitatively superior to the seedlings grown in the control soil.

The principle underlying CO2Fixator is the synergistic effect of bacteria and the fungus Trichoderma viride. This synergistic effect allows for CO2 to be fixed in the soil, contributing to the restoration of the soil. Each bacterium in CO2Fixator has specific metabolic capabilities and utilizes only the substrate that provides nutrients while releasing molecules that have antibiotic properties into the surrounding environment, preventing other bacteria from replicating and spreading and thereby promoting plant growth. Although Bacillus subtilis and Bacillus licheniformis produce CO2 as a byproduct 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 CO2Fixator ensures that the substrate is digested and metabolized into simple, ready-to-use components, promoting mutual nutrient supply. The other bacteria in CO2Fixator produce CO2 only as a secondary byproduct and only under particular physical–chemical conditions. CO2Fixator synergistically promotes soil restoration by preventing erosion, enhancing water retention, preventing soil leaching and the loss of both mineral salts and the organic matrix, and increasing soil volume, which lead to increases in soil fertility. Trichoderma viride regulates the vegetative processes of plants, promoting the absorption of nutrients after the bacterial mixture composts the substrate. The effectiveness of the preparation suggests that its use is not limited to the digestion of soil organic matter but could be extended to other types of organic substances, such as manure or sewage sludge. The quantity and quality of the organic substances digested could be the subject of further studies. More organic matter was present in the treated samples than in the control samples, which occurred due to a series of factors that, together, constitute a synergistic pathway. This pathway not only reduces CO2 emissions but also favours the development of additional plants that absorb other CO2, making the soil an ideal carbon sink. This study provides evidence related to improvements in soil quality, supporting the sustainability of agricultural practices. In addition to the increase in soil organic and inorganic components, with a consequent increase in soil volume, an improvement in the soil water retention capacity was also observed. The bioavailability of nitrogen and phosphorus, together with the ability of the soil to provide water and the action of Trichoderma viride, which favour rooting, allows quality soil to be obtained without the addition of additional substances. However, it is important to note that potassium should be added to the mixture to obtain the maximum synergistic effect and support bioaugmentation. CO2Fixator transforms agricultural soil into a carbon sink and improves soil quality. Compared with other techniques and systems of CO2 capture, such as reforestation, algae cultivation, air filtration or conversion of CO2 into fuel, CO2Fixator can be applied anywhere there is land, including to organic waste, sewage sludge and organic fertilizer. In the case of agricultural soil, bacterial digestion leads to the bioaugmentation of soil components, resulting in soil regeneration and making the soil progressively more fertile 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. 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 equivalents, were approximately 418 million tonnes. The widespread adoption of CO2Fixator could help offset 94% of Italy's total emissions.


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## Competing Interests

The authors have no relevant financial or nonfinancial interests to disclose.

## Author Contributions

SS designed the study, performed the experiments, analysed the data, and wrote the manuscript.

## Data Availability

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request

## References


1. Mahapatra, S., Yadav, R., & Ramakrishna, W. (2022). Bacillus subtilis impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. Journal of applied microbiology, 132(5), 3543–3562. https://doi.org/10.1111/jam.15480
2. Todorova, S., & Kozhuharova, L. (2010). Characteristics and antimicrobial activity of Bacillus subtilis strains isolated from soil. World journal of microbiology & biotechnology, 26(7), 1207–1216. https://doi.org/10.1007/s11274-009-0290-1
3. Sun, B., Gu, L., Bao, L., Zhang, S., Wei, Y., Bai, Z., …& Zhuang, X. (2020). Application of biofertilizer containing Bacillus subtilis reduced the nitrogen loss in agricultural soil. Soil Biology and Biochemistry, 148, 107911. https://doi.org/10.1016/j.soilbio.2020.107911
4. Chang, Y., Zhou, K., Yang, T., Zhao, X., Li, R., Li, J., …& Wei, Y. (2023). Bacillus licheniformis inoculation promoted humification process for kitchen waste composting: Organic components transformation and bacterial metabolic mechanism. Environmental research, 237(Pt 2), 117016. https://doi.org/10.1016/j.envres.2023.117016
5. Su, J., Zhou, K., Chen, W., Xu, S., Feng, Z., Chang, Y., … & Wei, Y. (2024). Enhanced organic degradation and microbial community cooperation by inoculating Bacillus licheniformis in low temperature composting. Journal of environmental sciences (China), 143, 189–200. https://doi.org/10.1016/j.jes.2023.08.037
6. 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.tb00433.x
7. 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
8. Su, J., Xue, Y., Zhang, K., Liu, Z., Lv, J., Yang, Q., …& Xin, H. (2024). The Effects of Lactobacillus plantarum, Bacillus subtilis, a Lignocellulolytic Enzyme System, and Their Combination on the Fermentation Profiles, Chemical Composition, Bacterial Community, and In Situ Rumen Digestion of Fresh Waxy Corn Stalk Silage. Animals: an open access journal from MDPI, 14(23), 3442. https://doi.org/10.3390/ani14233442
9. Claus, G., & Kutzner, H. J. (1985). Physiology and kinetics of autotrophic denitrification by Thiobacillus denitrificans. Applied Microbiology and Biotechnology, 22, 283-288. DOI:10.1007/BF00252031
10. Contreras-Cornejo, H. A., Macías-Rodríguez, L., del-Val, E., & Larsen, J. (2016). Ecological functions of Trichoderma spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS microbiology ecology, 92(4), fiw036. https://doi.org/10.1093/femsec/fiw036