

QUANTIFICATION OF SOIL ORGANIC CARBON AND MICROBIAL BIOMASS CARBON AND NITROGEN AT THE DEMETER AND ECOLAND CERTIFIED FARM, SONNENHOF, WOLPERTSHAUSEN (GERMANY)

Cristina PRUNĂ (BÜHLER)¹, Ramona Aida PĂUNESCU², Richard Dietmar BÜHLER³
Gabriela PĂUNESCU⁴, Aurel Liviu OLARU²

¹University of Craiova, Doctoral School of Animal and Plant Resources Engineering (IRAV), 13 A.I. Cuza Street, Craiova, Romania
author email: cristina.buehler@besh.de

²University of Craiova, 19 Libertății street, Craiova, Romania
author email: aida.paunescu@edu.ucv.ro; aurel.olaru@edu.ucv.ro

³Schwäbisch Hall AG Farmers' Haller Straße 2074549 Wolpertshausen, Germany
author email: stark.richard.i9n@student.ucv.ro

⁴University of Craiova, SCDA Caracal, 106 Vasile Alecsandri Street, Caracal, Romania
author email: paunescucraiova@yahoo.com

Corresponding author email: aida.paunescu@edu.ucv.ro

Abstract

This study quantifies soil organic carbon (SOC), total nitrogen (TN), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) in a biodynamic Demeter and Ecoland-certified farm located in Sonnenhof, Wolpertshausen (Germany). Soil samples were collected from six crop fields at three depth intervals (0–10, 10–20, and 20–30 cm) under different crop rotations: mustard–spelt wheat–alfalfa, lentils + camelina–coriander–alfalfa, mustard–spelt wheat–coriander, coriander–lentils + camelina–oriental mustard, mustard–coriander–oriental mustard, and alfalfa–alfalfa–spelt wheat.

The results revealed a clear depth-dependent decline of SOC, TN, MBC, and MBN across all sites, with the highest concentrations recorded in surface layers. Among rotations, the alfalfa-based system (Rheinsberg Acker) showed the greatest enrichment in SOC (2.11%), TN (0.22%), MBC (346.52 mg C_{mic} kg⁻¹), and MBN (58.65 mg N_{mic} kg⁻¹), demonstrating the critical role of perennial legumes in carbon sequestration and nitrogen accumulation through biological nitrogen fixation and root biomass input. In contrast, mustard–coriander rotations exhibited the lowest values, emphasizing the limited contribution of short-cycle crops to soil fertility.

These findings underscore the importance of diversified crop rotations, particularly those incorporating legumes, in enhancing soil carbon pools, supporting microbial activity, and promoting sustainable agricultural systems aligned with climate change mitigation objectives.

Key words: soil organic carbon, microbial biomass, nitrogen, crop rotation, sustainable agriculture

INTRODUCTION

Carbon dioxide (CO₂), the most significant greenhouse gas, accounts for approximately 60% of the total greenhouse effect contributing to global warming (Rastogi et al., 2002). Projections from various international studies indicate that, depending on the calculation methods

applied, atmospheric CO₂ concentrations may rise to between 490 and 1370 ppm by the year 2100 (Keidel et al., 2015). Kanemoto and colleagues (2016) observed that for most developed countries, the carbon footprint has increased significantly; for instance, since 1970, the United States' territorial carbon footprint rose by 23%,

consumption-based emissions by 38%, and the spatial footprint—defined as the minimum area required to contain 90% of emissions—by nearly 200%.

The agricultural sector contributes substantially to global carbon emissions through the production and use of agricultural machinery, and chemical crop protection agents such as herbicides, insecticides, fungicides, and fertilizers. Agriculture accounts for about 8% of the global carbon footprint, of which approximately 75% is directly linked to fertilizer use (Choudrie et al., 2008).

The sequestration of atmospheric CO₂ has become essential due to its rising concentration. The increase in CO₂ emissions, along with global warming and environmental degradation, is primarily driven by the global demand for energy (EPA Final Report, 2008). Compared to an estimated 300 Pg C emitted between 1850 and 2000, total emissions in the 21st century are projected to reach 950–2195 Pg, with an annual rate of 20–35 Pg C y⁻¹. Reducing CO₂ emissions can be achieved through biological, chemical, and technological methods aimed at both reduction and sequestration (Lal, 2008).

Soil organic carbon (SOC) plays a crucial role in maintaining soil health, supporting agricultural productivity, and mitigating climate change. As a major sink for atmospheric CO₂, SOC directly contributes to global carbon mitigation efforts. Understanding the spatial distribution and sequestration potential of SOC is therefore essential for sustainable land management. In recent years, interest has grown in how land-use practices influence SOC dynamics. Sustainable farming systems—

such as organic, regenerative, and agroecological approaches—have been shown to enhance SOC sequestration. However, significant uncertainties remain regarding SOC variation across land-use types and soil depths, particularly within organic agricultural systems where soil management strategies differ from conventional practices.

MATERIALS AND METHODS

The experiment was conducted in collaboration with the Akademie für Ökologische Land- und Ernährungswirtschaft Schloss Kirchberg an der Jagst and the Department of Soil Biology at the University of Hohenheim, Stuttgart. The research aims to quantify the variation of soil carbon at three depth intervals (0–10 cm, 10–20 cm, and 20–30 cm) and to evaluate the effects of crop rotation and minimum tillage practices implemented over the past three years on soil carbon dynamics during the 2023–2025 period. The main objective of this study is to investigate the distribution of soil organic carbon (SOC) and microbial biomass carbon (MBC) within the certified Sonnenhof Wolpertshausen farm, located in the Hohenlohe region of Germany.

The study was carried out across several crop fields within the biodynamic (Demeter) and organic (Ecoland-certified) farm at Sonnenhof Wolpertshausen, situated in the Hohenlohe region of Germany, at an altitude of 439 m above sea level (49.1675° N latitude and 9.8447° E longitude). Field sampling and analyses were performed during the 2025 growing season, following standardized agroecological research protocols for soil carbon assessment.

Table 1. Geographical location of the two farms

Country/Region	Location name	Latitude	Longitude	Altitude
Germany/ Hohenlohe (Wolpertshausen)	Plot Sällische Ost1	49.173891° N	9.853734° E	421.27 ± 3.00
	Plot Sällische West	49.173874° N	9.853698° E	423.06 ± 3.00
	Plot sor Wasserturm	49.170034° N	9.862211° E	450.62 ± 3.00
	Rudelsdorf Acker	49.168691° N	9.867321° E	450.51 ± 3.00
	Salat Acker	49.163144° N	9.867997° E	430.97 ± 3.00
	Sonnenhof Rheinsberg	49.154372° N	9.850831° E	428.08 ± 3.00

Table 2. List of crops with soil sampling depth

Year	Crops				
2025	Lentil + Camelina	Spelt wheat	Mustard	Coriander	Alfalfa
Soil depth	0-10cm 0-20cm 0-30cm	0-10cm 0- 20cm 0- 30cm	0-10cm 0- 20cm 0- 30cm	0-10cm 0-20cm 0-30cm	0-10cm 0-20cm 0-30cm



(a) Plot Sällische Ost1



(b) Plot Sällische West



(c) Plot sor Wasserturm



(d) Rudelsdorf Acker



(e) Salat Acker



(f) Sonnenhof Rheinsberg

Photo 1. A view of the different cultivated fields at the Sonnenhof Wolpertshausen farm, Germany, in 2025 (a–f).

RESULTS AND DISCUSSIONS

In this study, soil samples were systematically collected from crop fields in farm located in Germany, with three replications at each site and at three depth intervals (0-10 cm, 10-20 cm, and 20-30 cm). The objective was to investigate the distribution of soil organic carbon (SOC), total nitrogen (TN), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN). Six crop fields under a three-year crop rotation system (2023–2025) were selected. The crop rotations were: Parcela Sällisch Ost (Mustard-Spelt Wheat-Alfalfa), Parcela Sällisch West (Lentils+Camelina-Coriander-Alfalfa), Parcela Sor Wasserturm (Mustard-Spelt Wheat-Coriander), Rudelsdorf (Coriander-Lentils+Camelina-Oriental mustard), Salatacker (Mustard-Coriander-Oriental mustard), and Rheinsberg Acker (Alfalfa-Alfalfa-Spelt wheat).

Across all fields, SOC and TN were consistently highest in the upper soil layers (0-10 cm and 10-20 cm) compared to the 20-30 cm depth (Tables 3). Similarly, MBC and MBN showed depth-dependent pattern, with higher concentrations in surface soils and progressively lower values at greater depths (Tables 4).

Field 1. Plot Sällisch Ost (Mustard-Spelt Wheat-Alfalfa)

SOC was highest at 10-20 cm (2.15%), followed by 0-10 cm layer (2.13%), and lowest at 20-30 cm (1.74%). TN, however, peaked in the surface layer (0.22%), then decreased with depth (0.21% at 10-20 cm and 0.17% at 20-30 cm). MBC was greatest in the 0-10 cm layer (303.76 mg Cmic kg⁻¹), slightly lower at 10-20 cm (288.00 mg Cmic kg⁻¹), and lowest value at 20–30 cm (225.19 mg Cmic kg⁻¹). MBN declined with depth as

well, ranging from 32.86 mg Nmic kg⁻¹ at 0-10 cm to 19.43 mg Nmic kg⁻¹ at 20-30 cm.

Field 2. Plot Sällisch West (Lentils+Camelina-Coriander-Alfalfa)

SOC was highest at 0-10 cm (1.83%), followed by 1.77% at 10-20 cm and 1.47% at 20-30 cm. TN showed a similar trend: 0.18% (0-10 cm), 0.17% (10-20 cm), and 0.14% (20-30 cm). MBC values declined with depth, from 338.36 mg Cmic kg⁻¹ at 0-10 cm to 250.00 mg Cmic kg⁻¹ at 20–30 cm. Interestingly, MBN peaked at 10-20 cm (34.45 mg Nmic kg⁻¹), was slightly lower at 0-10 cm (30.72 mg Nmic kg⁻¹), and reached its minimum at 20-30 cm (25.76 mg Nmic kg⁻¹).

Field 3. Plot Sor Wasserturm (Mustard-Spelt Wheat-Coriander)

SOC and TN were highest at 0-10 cm (2.02% and 0.19%), with steady declines to 1.73% SOC and 0.16% TN at 20–30 cm. MBC followed the same pattern (342.43 to 190.97 mg Cmic kg⁻¹), as did MBN (41.34 to 27.67 mg Nmic kg⁻¹).

Field 4. Rudelsdorf (Coriander-Lentils+Camelina-Oriental mustard)

SOC decreased from 1.74% at 0-10 cm to 1.24% at 20-30 cm. TN was stable at 0.16% in the top two layers but dropped to 0.12% at 20-30 cm. MBC values decreased markedly with depth (349.94 to 141.34 mg Cmic kg⁻¹), and MBN showed the same decline (44.15 to 16.35 mg Nmic kg⁻¹).

Field 5. Salatacker (Mustard-Coriander-Oriental mustard)

SOC was highest at 0-10 cm (1.73%), followed by 1.48% at 10-20 cm, and lowest at 20-30 cm (1.28%). TN showed a similar pattern, with values of 0.16%, 0.13% and 0.11% across the three depths. MBC was highest in the topsoil (199.33 mg Cmic kg⁻¹), decreased to 136.57 mg Cmic kg⁻¹ at 10-20 cm, and reached 129.76 mg Cmic kg⁻¹ at 20-30 cm. MBN peaked at 10-20 cm (31.96 mg Nmic kg⁻¹), followed by 0-10 cm

(30.61 mg Nmic kg⁻¹), and lowest at 20-30 cm (27.35 mg Nmic kg⁻¹).

Field 6. Rheinsberg Acker (Alfalfa-Alfalfa-Spelt wheat).

SOC was highest in the 0-10 cm layer (2.28%), followed by 2.17% at 10-20 cm and lowest at 20-30 cm (1.87%). TN showed a similar depth-dependent decline, with values of 0.24%, 0.23%, and 0.19%, respectively. MBC peaked at 0-10 cm (392.57 mg Cmic kg⁻¹), decreasing steadily Nmic kg⁻¹, respectively), under the alfalfa–alfalfa–spelt wheat rotation (Table 4).

to 291.17 mg Cmic kg⁻¹ at 20-30 cm. MBN showed a similar trend, from 62.93 mg Nmic kg⁻¹ (0-10 cm) at surface to 50.41 mg Nmic kg⁻¹ (20-30 cm) in the subsoil.

Overall, the highest mean values of Total Organic Carbon (TOC), Total Nitrogen (TN), Microbial Biomass Carbon (MBC), and Microbial Biomass Nitrogen (MBN) were recorded in Rheinsberg Acker (2.11%, 0.22%, 346.52 mg Cmic kg⁻¹ and 58.65 mg

Table 3. Soil organic carbon (SOC), total nitrogen (TN), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) at three soil depths (0–10, 10–20, and 20–30 cm) across crop fields in the Germany Organic Ecoland Demeter-Certified Farm (2025)

Field no.	Field Name	Crop (2023)	Crop (2024)	Crop (2025)	Depth (cm)	Carbon Mean (%)	Nitrogen Mean (%)	Microbial Carbon Biomass (mg C _{mic} kg ⁻¹ soil)	Microbial Nitrogen Biomass (mg N _{mic} kg ⁻¹ soil)
1	Plot Sällisch Ost	Mustard	Spelt wheat	Alfalfa	0-10 cm	2.13	0.22	303.76	32.86
					10-20 cm	2.15	0.21	288.00	19.97
					20-30 cm	1.74	0.17	225.19	19.43
2	Plot Sällisch West	Lentils + Camelina	Coriander	Alfalfa	0-10 cm	1.83	0.18	338.36	30.72
					10-20 cm	1.77	0.17	304.14	34.45
					20-30 cm	1.47	0.14	250.00	25.76
3	Plot sor Wasserturm	Mustard	Spelt wheat	Coriander	0-10 cm	2.02	0.19	342.43	41.34
					10-20 cm	1.93	0.18	271.22	33.72
					20-30 cm	1.73	0.16	190.97	27.67
4	Rudelsdorf	Coriander	Lentils + Camelina	Oriental mustard	0-10 cm	1.74	0.16	349.94	44.15
					10-20 cm	1.66	0.16	244.89	37.03
					20-30 cm	1.24	0.12	141.34	16.35
5	Salatacker	Mustard	Coriander	Oriental mustard	0-10 cm	1.73	0.16	199.33	30.61
					10-20 cm	1.48	0.13	136.57	31.96
					20-30 cm	1.28	0.11	129.76	27.35
6	Rheinsberg Acker	Alfalfa	Alfalfa	Spelt wheat	0-10 cm	2.28	0.24	392.57	62.93
					10-20 cm	2.17	0.23	355.81	62.63
					20-30 cm	1.87	0.19	291.17	50.41

Table 4. Mean soil organic carbon (SOC), total nitrogen (TN), microbial biomass carbon (MBC), and microbial biomass nitrogen (MBN) averaged across six crop fields in the Germany Organic Ecoland Demeter-Certified Farm (2025).

Field	Crop (2023)	Crop (2024)	Crop (2025)	Total Carbon Mean (%)	Total Nitrogen Mean (%)	Microbial Carbon Biomass (mg C _{mic} kg ⁻¹ soil)	Microbial Nitrogen Biomass (mg N _{mic} kg ⁻¹ soil)
Plot Sällisch Ost	Mustard	Spelt wheat	Alfalfa	2.01	0.20	272.32	24.09
Plot Sällisch West	Lentils + Camelina	Coriander	Alfalfa	1.69	0.16	297.50	30.31
Plot sor Wasserturm	Mustard	Spelt wheat	Coriander	1.89	0.18	268.21	34.25
Rudelsdorf	Coriander	Lentils + Camelina	Oriental mustard	1.54	0.15	245.39	32.51
Salatacker	Mustard	Coriander	Oriental mustard	1.50	0.13	155.22	29.97
Rheinsberg Acker	Alfalfa	Alfalfa	Spelt wheat	2.11	0.22	346.52	58.65

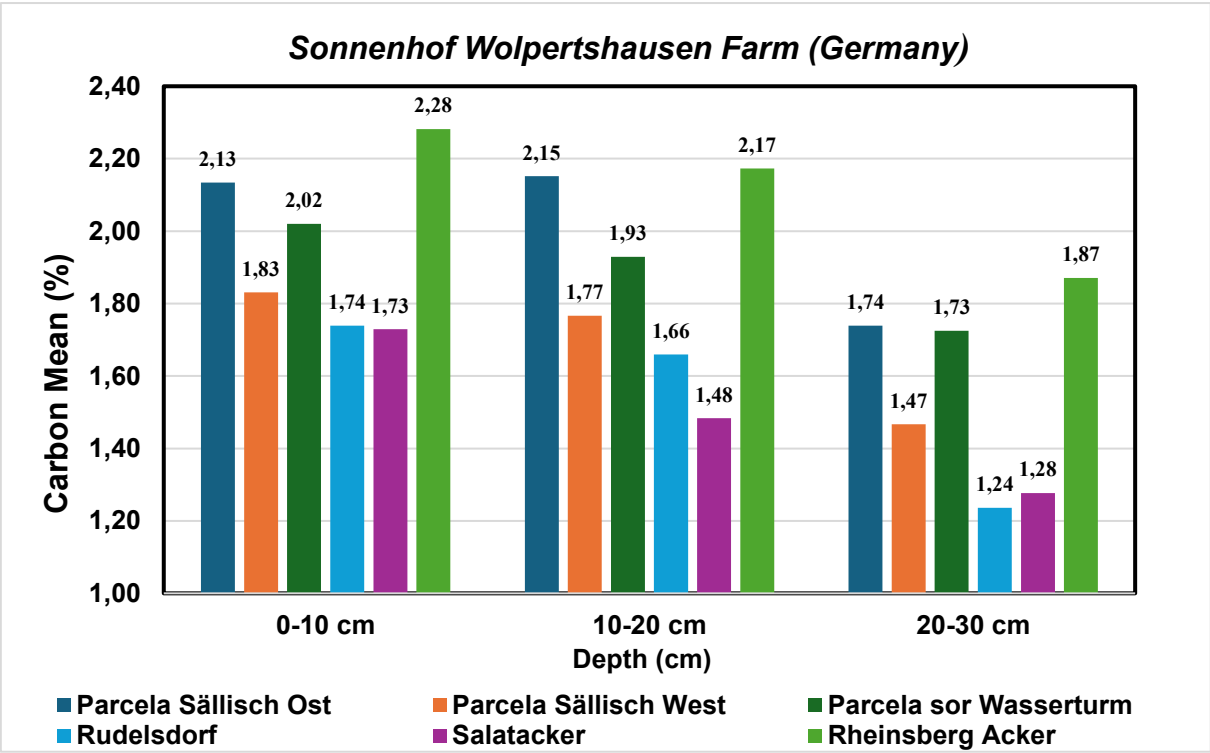


Figure 1. Depth-wise distribution of soil organic carbon (SOC) across six crop fields in the Germany Organic Ecoland Demeter-Certified Farm (2025).

This finding highlights the positive role of perennial alfalfa and legumes in enhancing both carbon and Nitrogen pool, largely due to their extensive root biomass and biological nitrogen fixation. In contrast, the

lowest value was observed in Salatacker (1.50%, 0.13%, 155.22 mg C_{mic} kg⁻¹ and 29.97 mg N_{mic} kg⁻¹, respectively), under a mustard–coriander–oriental mustard rotation. These results suggest that short-

cycle rotations with limited crop diversity and low organic inputs restrict microbial proliferation and reduce soil carbon–nitrogen accumulation.

These findings highlight the importance of crop rotation composition in shaping soil fertility. Legume-based rotations, particularly those including alfalfa, contributed substantially to soil organic carbon and nitrogen, likely due to biological nitrogen fixation, greater biomass inputs, and deeper root systems (Drinkwater et al., 1998; Peoples et al., 2009; Cong et al., 2015). This comparatively low soil fertility may be attributed to the dominance of mustard and coriander, which typically contribute less organic matter and have shorter, less extensive root systems compared to legumes (Blanco-Canqui and Lal, 2007). Overall, these results suggest that crop diversification with legumes is a key strategy for maintaining and enhancing soil carbon stocks and microbial activity (Kallenbach et al., 2016; Bowles et al., 2020).

CONCLUSIONS

Both SOC and MBC decreased significantly with depth across all sites. The sharpest decline was seen in Rudelsdorf and Salatacker, where SOC dropped by more than 60% between 0–10 cm and 20–30 cm. Rheinsberg Acker, however, retained comparatively higher SOC and MBC at depth, likely due to the persistent root system of alfalfa, which channels carbon deeper into the soil profile. This suggests that perennial legumes play a critical role not only in surface carbon enrichment but also in subsoil stabilization. The results highlight that crop rotation choice strongly influences SOC and MBC dynamics, with perennial legumes such as alfalfa exerting the most positive effect. Systems dominated by short-cycle annuals

like mustard and coriander appear less effective in maintaining soil carbon stocks, particularly in the subsoil.

REFERENCES

- Blanco-Canqui, H., Lal, R. (2007). Soil structure and organic carbon relationships following 10 years of wheat straw management in no-till. *Soil & Tillage Research*, 95(1–2), 240–254. <https://doi.org/10.1016/j.still.2006.12.005>.
- Bowles, T. M., Atallah, S. S., Campbell, E. E., Gaudin, A. C. M., Wieder, W. R., & Grandy, A. S. (2020). Long-term evidence shows that crop-rotation diversification increases agricultural resilience to adverse growing conditions in North America. *One Earth*, 2(3), 284–293.
- Choudrie, S. L., Jackson, J., Watterson, J. D., Murrells, T., Passant, N., Thompson, A., Cardenas, L., Leech, A., Mobbs, D. C., Thistlethwaite, G., Abbott, J., Dore, C., Goodwin, J., Hobson, M., Li, Y., Manning, A., Ruddock, K., & Walker, C. (2008). UK Greenhouse Gas Inventory, 1990 to 2006: Annual report for submission under the Framework Convention on Climate Change. *AEAT/ENV/R/2595 Issue 2. UK Department for Environment, Food and Rural Affairs*. Retrieved from <http://www.airquality.co.uk/archive/>.
- Cong, H., Zhang, M., Chen, Y., Chen, K., Hao, Y., Zhao, Y., & Feng, L. (2015). Highly selective CO₂ capture by nitrogen-enriched porous carbons. *Carbon*, 92, 297–304.
- Drinkwater, L. E., Wagoner, P., Sarrantonio, M. (1998). Legume-based cropping systems have reduced carbon and nitrogen losses. *Nature*, 396(6708), 262–265. <https://doi.org/10.1038/24376>.
- Kallenbach, C. M., Frey, S. D., & Grandy, A. S. (2016). Direct evidence for microbial-derived soil organic matter formation and its ecophysiological controls. *Nature*

- Communications*, 7, 13630.
<https://doi.org/10.1038/ncomms13630>.
- Kanemoto, K., Moran, D., Hertwich, E. (2016). Mapping the carbon footprint of nations. *Environmental Science & Technology*, 50(19), 10512–10517.
<https://doi.org/10.1021/acs.est.6b03227>.
- Keidel, L., Kammann, C., Grünhage, L., Moser, G., Müller, C. (2015). Positive feedback of elevated CO₂ on soil respiration in late autumn and winter. *Biogeosciences*, 12(4), 1257–1269.
<https://doi.org/10.5194/bg-12-1257-2015>
- Lal, R. (2008). Sequestration of atmospheric CO₂ in global carbon pools. *Energy & Environmental Science*, 1(1), 86–100.
<https://doi.org/10.1039/B809492F>
- Peoples, M. B., Unkovich, M. J., Herridge, D. F. (2009). Measuring symbiotic nitrogen fixation by legumes. In M. J. Unkovich (Ed.), *Nitrogen fixation in crop production* (pp. 125–170). *American Society of Agronomy*.
<https://doi.org/10.2134/agronmonogr52.c5>
- Rastogi, M., Singh, S., & Pathak, H. (2002). Emission of carbon dioxide from soil. *Current Science*, 82(5), 510–517.