

**TITLE :**

**CARBIOSOL: Biological indicators of soil quality and organic carbon in grasslands and croplands in Wallonia, Belgium**

**AUTHORS :**

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## **ABSTRACT:**

The protection of agricultural soil quality is critical to environmental sustainability and requires relevant indicators. Total soil organic carbon (SOC) is of importance for soil quality but its slow dynamic and inherent variability do not allow early detection of changes. The project CARBIOSOL provides a dataset from agricultural soils in Wallonia (Southern Belgium), of total SOC, SOC fractions and biological indicators, selected for their relevance as indicators of soil quality. Two land-uses (sampled in 2013), 5 agricultural region (2015), seasonal variability in croplands (2016) and four management types (2017) were studied. Soil organic carbon content (total, stable fine fraction  $<20 \mu\text{m}$ , labile coarse fraction  $>20 \mu\text{m}$ ), cold and hot water extractable carbon and nitrogen contents, total nitrogen,  $\text{pH}_{\text{KCl}}$ ,  $\text{pH}_{\text{H}_2\text{O}}$ , potential respiration, microbial biomass carbon and nitrogen, net nitrogen mineralisation, metabolic potential of soil bacteria, earthworm density and biomass, and two eco-physiological quotients (metabolic and microbial quotient) were measured for a total of 415 samples. The present dataset provides an important contribution for establishing a reference system of soil quality in Wallonia and eventually for large-scale studies through its integration into a global database. Moreover, the present dataset could be used to support the interpretation of measurements of fractions of SOC and biological indicators by soil analyses laboratories, which will be useful for farmers and decision makers to evaluate the effect of different management practices. Information contained in this publication or product may be reproduced, in part or in whole, and by any means for personal or public non-commercial uses, without charge or further permission, unless otherwise specified. Users are required to exercise due diligence in ensuring the accuracy of the material reproduced, indicate the complete title of the material produced and refer to this publication (including author names), indicate that the reproduction is a copy/uses official work financed by the SPW-DGO3. Commercial reproduction and distribution is prohibited, except with written permission from SPW-DGO3 and publication authors.

## **Keywords:**

Database, Biological indicators, Carbon fractions, Soil quality, Agricultural soil, Soil Monitoring Network, Wallonia, Cropland, Grassland, Soil Microbial Biomass, Soil Organic Matter.

## **INTRODUCTION:**

Soils contribute to basic human needs, such as the provision of food, regulation of water and air quality, and they are a major reserve of biodiversity (Keesstra *et al.*, 2016). Furthermore, soils have been recognized as a non-renewable resource within a human lifespan (Lal 2009) and soil quality is affected by human actions, especially agricultural practices. Indeed, agricultural intensification has led to soil degradation and the loss of natural habitats (Foley *et al.*, 2005), soil biodiversity (Tsiafouli *et al.*, 2015) and regulatory functions (MEA 2005, Kremen and Miles 2012, Duru *et al.*, 2015). Hence, indicators for soil quality assessment are needed to evaluate the effects of agricultural practices, for an early detection of the degradation of soil quality, and to support sustainable soil management.

Among these indicators, total soil organic carbon and carbon fractions are relevant to relate the different forms of soil organic matter to their functional roles in soil (Dalal and Chan, 2001). However, evolution of total soil carbon is slow and does not allow to assess early perturbations. Carbon fractions might provide more relevant data for assessment of soil quality changes in response to agricultural management. In contrast, living organisms are early indicators of changes in soil quality, informing both on soil perturbations (like management practices; D'Hose *et al.*, 2014; van Leeuwen *et al.*, 2015) and functioning (Bunning and Jiménez 2003). Although several monitoring networks exist across Europe (van Leeuwen *et al.*, 2017), none of them take both biological indicators and carbon fractions into account.

In Wallonia (Southern Belgium), data on carbon fractions and biological indicators from agricultural soils were so far not available. The project CARBIOSOL « Organic carbon, microbial biomass and

activity of soils: towards an indicator of soil quality in Wallonia (Belgium) » was then initiated (Krüger *et al.*, 2017). This project aimed at integrating biological indicators and soil carbon fractions for cropland and grassland soils into CARBOSOL, a monitoring network of soil organic carbon stocks and dynamics in Wallonia (Goidts and van Wesemael, 2007). The CARBOSOL network was based on a selection of sites from the 13033 sites from Aardewerk project (1949-1965; Van Orshoven *et al.*, 1988), established for soil mapping in Belgium. CARBOSOL network consisted of a random selection of 434 sites from Aardewerk, within 15 landscape units (LSU, defined according to soil type, land use and agricultural region (Goidts, 2009)), representing 64% of the useful agricultural surface of Wallonia. The database CARBIOSOL was compiled from measurements performed with the same methods and sampling scheme, within selected sites from CARBOSOL (see below). Samplings were performed in different years, corresponding to studies focusing on a specific question: differences between two land-uses (in 2013 and 2014), five agricultural regions (in 2015) (DGO3, 2017), four sampling seasons (in 2016), seven crop types (in 2017) and four land managements (in 2017). Hence, soil organic carbon content (as total, fine fraction <20  $\mu\text{m}$  and coarse fraction >20  $\mu\text{m}$ ), cold and hot water extractable carbon and nitrogen content, total nitrogen,  $\text{pH}_{\text{KCl}}$ ,  $\text{pH}_{\text{H}_2\text{O}}$ , potential respiration, microbial biomass carbon and nitrogen, net nitrogen mineralisation, metabolic potential of soil bacteria, earthworm density and biomass, and two eco-physiological quotients (metabolic and microbial quotient) were measured for a total of 415 samples in 124 cropland and 66 grassland sites, respectively. Both carbon fractions and biological indicators are available for 172 samples. This heterogeneity of the database needs to be taken into account for analyses, as the presence of data for specific variables is dependent on the year of study.

Such indicators are increasingly in demand in Wallonia and require the establishment of reference systems to provide a ‘baseline’ or ‘normal’ values (Nortcliff, 2002). The present dataset provides a basis for a future reference system for soil quality assessment in Wallonia (Krüger *et al.*, 2018b). Furthermore, this dataset could be used for large-scale comparison of soil quality indicators and be included in a larger database like the European Soil Database (Morvan *et al.*, 2008, Pulleman *et al.*, 2012). Finally, in order to offer these measurements as support for practical advice to farmers, soil analyses laboratories require large datasets of indicators (Doran and Zeiss 2000). Hence, the present dataset provides a first reference system in Wallonia, supporting the implementation of measurements of carbon fractions and biological indicators as routine analyses in these laboratories.

## **METADATA:**

### **Class I. Data set descriptors**

#### **A. Data set identity**

**Title: CARBIOSOL: Biological indicators of soil quality and organic carbon in grasslands and croplands in Wallonia, Belgium**

#### **B. Data set identification code**

**Suggested data set identity codes: DB\_CARBIOSOL.csv**

#### **C. Data set description**

##### **1. Originators:**

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## 2. Abstract:

The protection of agricultural soil quality is critical to environmental sustainability and requires relevant indicators. Total soil organic carbon (SOC) is of importance for soil quality but its slow dynamic and inherent variability do not allow early detection of changes. The project CARBIOSOL provides a dataset from agricultural soils in Wallonia (Southern Belgium), of total SOC, SOC fractions and biological indicators, selected for their relevance as indicators of soil quality. Two land-uses (sampled in 2013), 10 landscape units (2015), seasonal variability in croplands (2016) and four management types (2017) were studied. Soil organic carbon content (total, stable fine fraction  $<20\ \mu\text{m}$ , labile coarse fraction  $>20\ \mu\text{m}$ ), cold and hot water extractable carbon and nitrogen contents, total nitrogen,  $\text{pH}_{\text{KCl}}$ ,  $\text{pH}_{\text{H}_2\text{O}}$ , potential respiration, microbial biomass carbon and nitrogen, net nitrogen mineralisation, metabolic potential of soil bacteria, earthworm density and biomass, and two eco-physiological quotients (metabolic and microbial quotient) were measured for a total of 415 samples. The present dataset provides an important contribution for establishing a reference system of soil quality in Wallonia and eventually for large-scale studies through its integration into a global database. Moreover, the present dataset could be used to support the interpretation of measurements of fractions of SOC and biological indicators by soil analyses laboratories, which will be useful for farmers and decision makers to evaluate the effect of different management practices. Information contained in this publication or product may be reproduced, in part or in whole, and by any means for personal or public non-commercial uses, without charge or further permission, unless otherwise specified. Users are required to exercise due diligence in ensuring the accuracy of the material reproduced, indicate the complete title of the material produced and refer to this publication (including author names), indicate that the reproduction is a copy/uses official work financed by the SPW-DGO3. Commercial reproduction and distribution is prohibited, except with written permission from SPW-DGO3 and publication authors.

## **D. Key words**

Database, Biological indicators, Carbon fractions, Soil quality, Agricultural soil, Soil Monitoring Network, Wallonia, Cropland, Grassland, Soil Microbial Biomass, Soil Organic Matter.

## **Class II. Research origin descriptors**

### **A. Overall project description**

#### **1. Identity:**

The project CARBIOSOL:

« Organic carbon, microbial biomass and activity of soils: towards an indicator of soil quality in Wallonia ». ‘Carbone organique, biomasse et activité microbienne des sols: vers un indicateur de la qualité des sols en Wallonie’ (15/5/2013-31/12/2014, Chartin *et al.*, 2015),

‘Carbone organique, biomasse et activité microbienne des sols: vers un indicateur de la qualité des sols en Wallonie (Carbiosol 2)’ (1/1/2015-30/11/2015, Krüger *et al.*, 2015),

‘Carbone organique, biomasse et activité microbienne des sols: vers une cartographie des indicateurs de la qualité des sols en Wallonie (Carbiosol 3):’ (1/12/2015-31/12/2016, Chartin *et al.*, 2016),

‘Développement d’indicateurs de la qualité biologique et du carbone organique du sol pour l’évaluation de l’état des sols en Wallonie (Carbiosol 4) (1/1/2017-31/1/2018, Krüger *et al.*, 2018a).

#### **2. Originators:**

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#### **3. Period of Study:**

2013-2018.

#### **4. Objective:**

The project CARBIOSOL « organic carbon, microbial biomass and activity of soils: towards an indicator of soil quality in Wallonia » aimed at expanding a Walloon soil monitoring network by integrating measurements of biological indicators and soil carbon fractions. The main goals of the project were to (1) select biological and carbon indicators for the assessment of soil quality in Wallonia, (2) establish ranges of values for those indicators, and (3) develop statistical, graphical and cartographic tools for end users.

#### **5. Abstract:**

Same as above.

#### **Sources of funding:**

This project was funded by the Public Administration of Wallonia (Service Public de Wallonie, SPW-DGO3).

### **B. Specific subproject description**

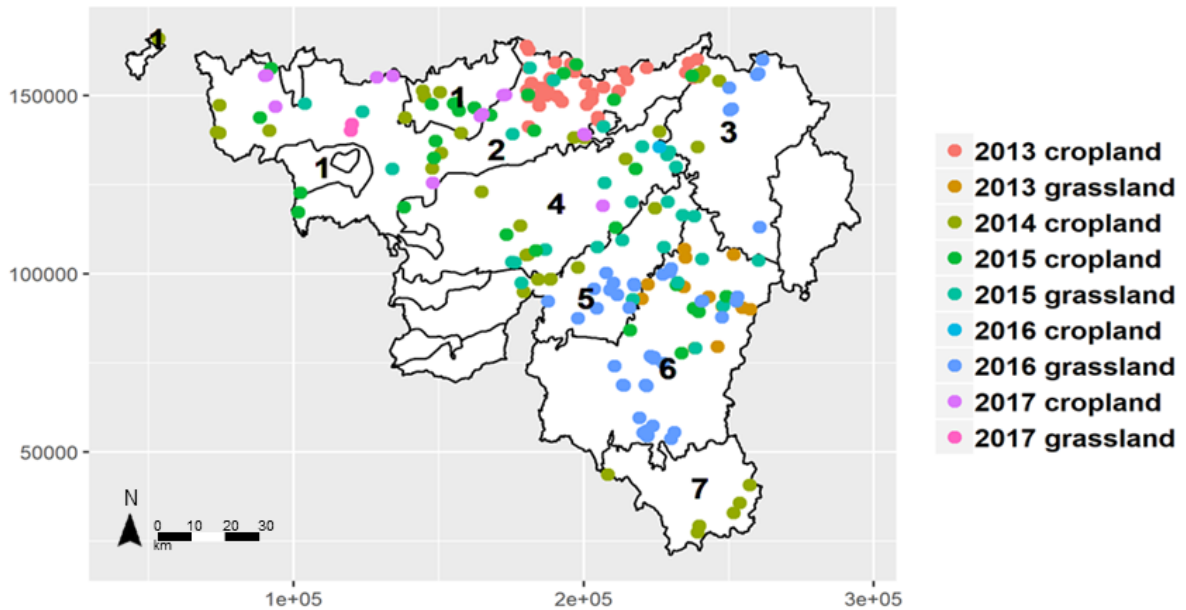
#### **B.1. Sampling campaigns:**

Overall, 415 samples were analysed from sampling campaigns in 2013-2014, 2015, 2016 and 2017 from a total of 190 sites (124 croplands, 66 grasslands) (Table 1). 309 samples from cropland and 106 samples from grassland were taken.

**Table 1: Number of sites sampled, according to agricultural region, land use and land management in the CARBIOSOL campaigns.**

Agricultural region	Land use	Land management	CARBIOSOL 1 (2013-2014)	CARBIOSOL 2 (2015)	CARBIOSOL 3 (2016)	CARBIOSOL 4 (2017)
ardenne	cropland	conventional		6		
	grassland	conservative			17	
		conventional	19	6	9	
condroz	cropland	organic			6	
		organic transition	6	6	16	4
	grassland	conventional		12		
famenne	cropland	conventional	6			
	grassland	conservative			7	
		conventional		6	3	
grassland	cropland	conventional	5			
high-ardenne	grassland	conservative			4	
		conventional			3	
jurassic	cropland	conventional	6			
loamy	cropland	conservative			16	12
		conventional	50	12	64	16
		organic			16	12
	organic transition				8	
grassland	conventional		6			
sandy-loamy	cropland	conservative			16	4
		conventional	6	6	16	4
		organic transition				4
<b>Total</b>			<b>98</b>	<b>60</b>	<b>193</b>	<b>64</b>

Earthworms counting was performed close to the soil sampled for laboratory analysis, but data were assigned to the same ‘sample identifier’. In 2013, 2014 and 2015, one composite sample was analysed at each site; in 2016 and 2017, four replicate composite samples were analysed at each site (see below). For all sampling campaigns, the composite samples consisted of five individual samples taken between one and four meters from a central point (localized in CARBIOSOL network) with a manual auger, following (Goidts, 2009). Five agricultural regions (DGO3, 2017) and eight different agricultural practices were studied during four campaigns (Fig.1). Geographic coordinates cannot be provided because sites are private properties (data protection privacy).



1:sandy-loamy, 2:loamy, 3:grassland, 4:condroz, 5:famenne, 6:ardenne, 7:jurassic

**Figure 1: Map of Wallonia (south Belgium) with the sampling sites (croplands and grasslands) of the CARBIOSOL project.**

Sampling campaign in autumn 2013 (October and November) and spring 2014 (March, April and May (Carbiosol 1): 98 sites, subjected to two land uses, were selected within the CARBIOSOL. A total of 19 samples of grassland soils in the Ardenne region and 79 soil samples under conventional agricultural management in the Loamy regions (carbon and biological indicators (Krüger *et al.*, 2017)) and in the Condroz, Famenne, Grassland, Jurassic, and Sandy-loamy regions (carbon only) were analysed. One composite sample was taken at each site. During this campaign, carbon and nitrogen from hot and cold-water extractions were not assessed (WSC, HWC, WSN and HWN respectively).

Sampling campaign in spring 2015 (Carbiosol 2): Within 10 main LSU (in terms of surface area) of CARBIOSOL, 60 sites were selected through a Latin hypercube method (Minasny and McBratney, 2006), according to soil texture and organic carbon content. 30 samples of grassland soils in the Ardenne, Condroz, Famenne and Loamy regions and 30 samples of soils under conventional agricultural management in the Ardenne, Condroz, Loamy and Sandy-loamy regions were assessed (Krüger *et al.*, 2018a). One composite sample was taken at each site.

Sampling campaigns in April, June, August and October 2016 (Carbiosol 3): the aim of this sampling campaign was to assess the seasonal variability of indicators in cropland soils only (Krüger *et al.*, 2018a). Cropland sites were selected according to current (winter wheat) and previous (sugar beet) crop, which represents the most common crop rotation in Wallonia. Four composite samples were taken in each angle of a 10 m square, located randomly, at each site. Samples were taken in two-monthly intervals (April, June, August, and October) at nine sites located in the Condroz, Loamy and Sandy-loamy regions. Thus, 144 samples were taken during these sampling campaigns. Six sites were under conventional management with winter wheat as current crop and sugar beet as previous crop. The other three sites were under different management: recently converted to organic farming, conservation agriculture including straw incorporation, a cover crop and no-till. Moreover, 15 grassland sites (49 samples), from Ardenne, Famenne and High Ardenne, were sampled in November 2016 to study the effects of different management: mowed (short or long-term) and grazed grassland.

Sampling campaign in June 2017 (Carbiosol 4): the aim of this campaign was to assess the effects of land management (conventional agriculture, conservation agriculture (no till or reduced tillage), conversion to organic farming, and organic farming) on soil quality indicators (Krüger *et al.*, 2018a). Sites were selected according to land management and farmer's willingness to participate in the study, after an enquiry performed by the authors in collaboration with a farmer's association (GREENOTEC). Four composite samples were taken in each angle of a 10 m square, located randomly, at each site. Samples were taken in 16 crop sites (including four paired sites), located in the Condroz, Loamy and Sandy-loamy regions. Site S2 is paired with S2b, S3 is paired with S3b, S4 is paired with S4b, S5 is paired with S5b. Moreover, croplands were covered with different crop types (beet, corn, lucerne/clover/fescue, pea, pepper, potato, wheat and short-term grassland). Thus, a total of 64 samples were taken during this sampling campaign.

## B.2. Sampling and analytical methods:

Composite soil samples consisted of five samples taken between one and four meters within a circle from a central point with a manual auger, following Goidts *et al.*, (2009). Soils were sampled at 0-10 cm depth, except for Corg and both carbon fractions (Corg\_abv20 and Corg\_lwr 20) in croplands which were sampled at 0-20 cm depth. When replicate samples were taken (in 2016 and 2017), the four individual composite samples were located at the corners of a 10 m square. For pH, hot and cold water extractions and biological indicators, fresh soil samples were sieved (4 mm), stored at 4 °C until analyses. Soil moisture was determined after drying at 105 °C for 3h (Allen *et al.*, 1989). Water holding capacity (WHC) of sieved soil was measured using a Haines-funnel system, where 50 ml of water was added to 50 g of fresh soil for 30 min. Excess water was collected and its volume measured (Jenkinson and Powlson, 1976). Before analyses of microbial activities (N mineralization, respiration), soil samples were adjusted to 50–60% WHC. For soil C, N and carbon fractionations, soils were air-dried, gently crushed using a pestle and mortar and sieved at 2 mm in agreement with the protocols for routine soil fertility analysis (Genot *et al.*, 2011). All analyses were performed within a month of soil sampling.

### ***Physico-chemical analyses***

pH was measured in a suspension (1:1; m:v) in 1 M KCl with a pH meter (HI2550 HANNA instruments, USA) (ISO 10390, 2005).

For C fractionation, 10 g of fine earth (< 2 mm) was mixed with 100 ml of deionized water in a plastic bottle and shaken horizontally for 15 minutes at 250 rpm in order to disrupt macro-aggregates. The fine earth-water mixture was then transferred to a 50 µm sieve and washed through into a beaker until the liquid that passed the sieve was clear. The material that passed through the 50 µm sieve was then poured on a 20 µm sieve and washed through using a spray bottle filled with deionized water and a rubber spatula until the liquid that passed the sieve was clear. The 50µm sieving step was included in order to prevent the clogging of the finer sieve (20 µm). Care was taken to keep the total amount of water used for shaking and washing below 2 l, in order to reduce losses during centrifugation and drying. The fractions remaining on the sieves were collected and dried at 60°C. The liquid with the fraction finer than 20 µm was centrifuged for 25 minutes at 3600 rpm and the clear supernatant liquid was discarded. The remainder was transferred into a beaker and dried at 60°C for minimum four days.

The fine earth (<2 mm) and the fine fraction (<20 µm) were analyzed for their concentration of organic carbon (Corg and Corg\_lwr20, respectively, in gC/100g). The C concentration of the coarse fraction (>20 µm) was calculated from its mass, and the mass and C concentrations of the fine earth and the fine fraction (Corg\_abv20 in gC/100g). First, the samples were tested for the presence of inorganic carbon (Ci) as calcium carbonate using a 5% HCl solution. If inorganic carbon was present, its content was determined by the calcimetric method using an electronic manometer (Sherrod *et al.*, 2002). Total carbon (Ct) and total nitrogen (Ntot) was analysed by dry combustion using a VarioMax CN Analyzer (Elementar GmbH, Germany). Finally, soil organic carbon (Corg and Corg\_lwr20) was calculated by



correcting for the inorganic carbon content ( $C_{org}$  or  $C_{org\_lwr20} = C_t - C_i$ ). More details in van Wesemael *et al.*, (2019).

### **Biological indicators**

Soil microbial biomass carbon (MBC) and nitrogen (MBN) were determined by the chloroform fumigation extraction method (Vance *et al.*, 1987). In extracts, dissolved organic carbon was measured with a Total Organic Carbon Analyzer (Labtoc, Pollution and Process Monitoring limited, UK) and total nitrogen was measured colorimetrically using a continuous flow analyzer equipped with a UV digestion unit (Autoanalyser3, BranLuebbe, Germany).

The respiration potential (RP, Robertson *et al.*, 1999) was measured as CO<sub>2</sub> accumulation in the headspace (250 ml) of an amber bottle (Supelco, USA) from 20 g fresh soil, at 15°C in the dark after an overnight pre-incubation. Gas samples (4 ml) were taken at 0, 120, 150, and 180min (samples taken in 2015) or 0, 120, 240, and 360 min (samples taken in 2016) with an air-tight syringe (Hamilton Model 1005) and analyzed with an infrared absorption gas analyzer (EGM-4, PPsystem, UK). The RP was estimated by linear regression of CO<sub>2</sub>-C against time ( $mg \cdot kg^{-1} \cdot h^{-1}$ ).

Net nitrogen mineralisation (N<sub>min</sub>) was measured through a 29-day aerobic laboratory incubation at constant temperature (25 °C) in the dark (Hart *et al.*, 1994). At the beginning and at the end of the incubation, inorganic nitrogen was extracted with a 1M KCl solution (1:5; w:v) (Allen *et al.*, 1989) and analysed colorimetrically using a continuous flow analyser (Auto- Analyser3, BranLuebbe, Germany). The N<sub>min</sub> rate was calculated by dividing the net increase in inorganic nitrogen (N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup>) during the incubation period by the number of incubation days.

BIOLOG ECOplates (BIOLOG™, California), with 32 wells, each containing 31 different carbon substrates and one negative control well with water, were used to assess metabolic potential (MP) of soil bacteria. Each well contained an oxidized tetrazolium dye, changing from colourless to purple when bacterial respiration oxidized the carbon source provided. 1 g fresh soil was extracted with 9 ml 0.1% sodium cholate and diluted (10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>) with 0.85% NaCl to determine the number of CFU (colony forming units). An aliquot of 100 μl of the dilution corresponding to 1000–2000 CFU was incubated in the ECOplates for 72h at 20 °C. The number of substrates used by bacteria was detected through visual observation of coloured wells after incubation (Buysse *et al.*, 2013).

The metabolic quotient (qCO<sub>2</sub>) represents the quantity of respired CO<sub>2</sub>-C per unit of soil microbial biomass and was calculated by dividing RP by soil MBC (Anderson and Domsch 1990). The microbial quotient (q<sub>mic</sub>) represents the availability of substrate for microorganisms and was calculated by dividing MBC by C<sub>org</sub> (Anderson and Domsch 1990).

One earthworm sample was collected at each site, except in spring 2014, close to the marked sites, but outside the sampling radius defined for soil samples. Thus, data for 326 earthworm samples are in the database. Earthworms were extracted by two consecutive applications of 4 l mustard solution (3 and 6 g.l<sup>-1</sup> of mustard, *Sinapis Albae Seminis Pulvis*, Pharmaflore, respectively) (Gunn 1992, Lawrence and Bowers 2002) on a 30×60 cm surface marked with a wooden frame. All earthworms leaving the soil were collected and conserved in 70% ethanol. Earthworms were counted and their mass measured. Earthworm density (EWD) was calculated by the number of earthworms collected divided by the surface of the frame and the earthworm mass (EWM) corresponds to the total earthworm biomass saturated with ethanol.

More details about biological indicators can be found in Krüger *et al.* (2018).

#### 4. Project personnel

##### **Principle investigators:**

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##### **Project leaders:**

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Monique Carnol, Laboratory of Plant and Microbial Ecology, InBioS, University of Liège, Botany Bât. 22, Chemin de la Vallée 4, 4000 Liège, Belgium; Email: m.carnol@uliege.be

##### Technicians :

M. Bravin, A. Degueldre, A. Piret, and M.-C. Requier

### **Class III. Data set status and accessibility**

#### **A. Status**

Latest updates : February 22, 2019

Data Archiving: Provided with the metadata file. Supporting Information for review and publication.

Latest archive date : February 22, 2019

Metadata status : Metadata are complete.

Data Verification: Data were reviewed and corrected for any errors.

#### **B. Accessibility**

Storage location and medium: The database is stored by ESA Ecology. Database is also stored on the server of the University of Liège (French version) and is accessible for data paper authors.

Contact person: Monique CARNOL (m.carnol@uliege.be)

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Costs: none.

## **Class IV. Data structural descriptors**

### **A. Data set file:**

Identity: CARBIOSOL: Biological indicators of soil quality and organic carbon in grasslands and croplands in Wallonia, Belgium

Size: 366 lines (excluding header row) and 32 columns.

Format and storage mode: Comma-Separated Values format, no compression.

Header information: Headers are given as variable names of each parameter (descriptive and analytics). They are with underscores to allow direct use in statistical software.

Alphanumeric attributes: mixed.

Special characters/fields: none.

### **B. Variable definitions**

Variable definitions are presented in table 2.

**Table 2: Description of the CARBIOSOL database variables**

Variable name	Description of variable	Unit	Variable codes	Description of variable codes
Project	project	/	carbiosol_1	first year of the project
			carbiosol_2	second year of the project
			carbiosol_3	third year of the project
			carbiosol_4	fourth year of the project
ID_sample	sample identifier	/	1-415	
Period_sampling	month and year of sampling	/	october-13	October 2013
			november-13	November 2013
			march-14	March 2014
			april-14	April 2014
			may-14	May 2014
			april-15	April 2015
			may-15	May 2015
			june-15	June 2015
			april-16	April 2016
			june-16	June 2016
			august-16	August 2016
			october-16	October 2016
			november-16	November 2016
			june-17	June 2017
			Year_sampling	year of sampling
2015	2015			
2016	2016			
2017	2017			
ID_site	site identification			

			NA	information not available
			S1-S11	
			S2 <sub>a/b</sub> -S5 <sub>a/b</sub>	farmers' sites
			S6	
			S9-S12	
			A_1a1-A_1a2	
			A_1b1-A_1b5	
			A_2b1-A_2b3	
			F_1b1	BIOECOSYS network sites
			F_1b3	
			F_1b4	
			H_1b1	
			H_1b4	
Replicate	replicate number	/	NA	information not available
			1-4	
Agricultural_region	agricultural regions of Wallonia, defined according to their natural characteristics and their agro-economic potential	/		
			ardenne	Ardenne
			condroz	Condroz
			famenne	Famenne
			grassland	Grassland
			jurassic	Jurassic
			loamy	Loamy
			sandy-loamy	Sandy-loamy
			high-ardenne	High Ardenne
Land_use	land use type	/		
			cropland	cropland
			grassland	grassland
Land_management	land management	/		

			conventional	conventional farming
			organic	organic farming
			organic_transition	under transition to organic farming
			conservative	conservative farming
Agricultural_practice	agricultural practice	/		
			SNIT	shallow non-inversion tillage
			NT_OT	no-till, occasional tillage farming
			mowed_short-term_grassland	mowed short-term grassland
			mowed_long-term_grassland	mowed long-term grassland
			grazed_grassland	grazed grassland
			NA	information not available
Crop	crop at time of sampling	/		
			beet	sugar beet ( <i>Beta vulgaris</i> )
			maize	maize ( <i>Zea mays</i> )
			luc_clov_fesc	lucerne, clover and fescue ( <i>Medicago sativa</i> , <i>Trifolium sp.</i> and <i>Festuca sp.</i> )
			pea	pea ( <i>Pisum sativum</i> )
			pumpkin	pumpkin ( <i>Cucurbita maxima</i> )
			potato	potato ( <i>Solanum tuberosum</i> )
			wheat	winter wheat ( <i>Triticum sp.</i> )
			short-term_grassland	short-term grassland
			NA	information not available
Year_transition	year of transition to organic farming	/		
			1991	1991
			2002	2002
			2009	2009
			2016	2016
			2017	2017
			NA	information not available
pHkcl	pH measured in KCl	/		
pHwater	pH measured in water	/		

Corg	total soil organic carbon content	gC/100g
Corg_lwr20	organic carbon content in soil fraction finer than 20 µm	gC/100g
Corg_abv20	organic carbon content in soil fraction coarser than 20 µm	gC/100g
WSC	water soluble carbon, cold water extractable carbon (20 °C)	mg C.kg <sup>-1</sup>
HWC	hot water extractable carbon (80 °C), "Labile carbon"	mg C.kg <sup>-1</sup>
WSN	water soluble nitrogen, cold water extractable nitrogen (20 °C)	mg N.kg <sup>-1</sup>
HWN	hot water extractable nitrogen (80 °C)	mg N.kg <sup>-1</sup>
Ntot	total soil nitrogen content	‰
Corg_Ntot	ratio between soil organic carbon and nitrogen contents	/
MBC	microbial biomass carbon	mg C.kg <sup>-1</sup>
MBN	microbial biomass nitrogen	mg N.kg <sup>-1</sup>
MBC_MBN	ratio between and microbial biomass nitrogen	/
RP	respiration potential (C mineralization rate)	mg C-CO <sub>2</sub> .kg <sup>-1</sup> .h <sup>-1</sup>
MP	metabolic potential of soil bacteria	%
Nmin	net nitrogen mineralisation	mg N.kg <sup>-1</sup> .day <sup>-1</sup>
qCO2	metabolic quotient=RP/MBC	mg C-CO <sub>2</sub> .kg <sup>-1</sup> C.h <sup>-1</sup>
qmic	microbial quotient=MBC/Corg	%
EWD	earthworm density	ind. m <sup>-2</sup>
EWM	earthworm mass	g. m <sup>-2</sup>

<sup>1</sup>: <http://etat.environnement.wallonie.be/contents/indicatorsheets/PHYS%205.html>

**Project (Project):**

Phase of the CARBIOSOL project, i.e. carbiosol\_1 (2013-2014), carbiosol\_2 (2015), carbiosol\_3 (2016), carbiosol\_4 (2017).

**Sample identification (ID\_sample):**

Unique ID number assigned to each sample.

**Period of sampling (Period\_sampling):**

Period of sampling, *i.e.* the month and the year.

**Year of sampling (Year\_sampling):**

Year of sampling.

**Site identification (ID\_site):**

Unique site identifier.

**Replicate (Replicate):**

Replicate number. Four replicate samples were taken at each site in 2016 and 2017. For other samples, one replicate was taken at each site.

**Agricultural region (Agricultural\_region) :**

Agricultural regions of Wallonia (Southern Belgium), defined according to their natural characteristics and their agro-economic potential. For more details on agricultural regions, see Goidts and van Wesemael, (2007), and in p.20 of state of environment report in Wallonia (DGO3, 2017).

**Land use (Land\_use):**

Type of land use, *i.e.* cropland or grassland.

**Land management (Land\_management):**

Type of land management *i.e.* conventional farming, organic farming, under transition to organic farming and conservation agriculture. Conservation agriculture is “a concept for resource-saving agricultural crop production that strives to achieve acceptable profits together with high and sustained production levels while concurrently conserving the environment” (FAO 2010). It is defined as minimal soil disturbance (no-till, for example) and permanent soil cover (mulch, for example), combined with rotations (Hobbs *et al.*, 2008). Sites were classified into ‘transition to organic farming’ when under organic farming below three years.

**Agricultural practice (Agricultural\_practice):**

Agricultural practice *i.e.* shallow non-inversion tillage, no-till or occasional tillage in crops and mowed (short or long-term) and grazed grassland.

**Crop (Crop):**

Crop type at time of sampling (beet, corn, wheat, for example).

**Year of transition (Year\_transition):**

Year of conversion from conventional to organic or conservation farming.

**pH<sub>KCl</sub>:**

pH measured in KCl.

**pH<sub>water</sub>:**

pH measured in distilled water.



**Soil organic carbon (Corg):**

Total soil organic carbon concentration.

**Coarse and fine fractions of soil organic carbon (Corg\_abv20 and Corg\_lwr20, respectively):**

These two fractions are obtained by physical fractionation of soil fine earth (<2mm) shaken for 15 min for macro-aggregates disruption (van Wesemael *et al.*, 2019). The coarse fraction is the organic carbon content in soil particles larger than 20  $\mu\text{m}$  (Corg\_abv20). It's a mixture of microaggregates, small macroaggregates and particulate organic matter and mainly contains organic carbon, available to microorganisms, and stabilized C in microaggregates >20 $\mu\text{m}$ . The fine fraction is the organic carbon content in the soil particles below 20  $\mu\text{m}$  (Corg\_lwr20). It contains free particles of fine clay and silt, and micro-aggregates <20 $\mu\text{m}$  where organic carbon is stabilised as organo-mineral complexes (Hassink 1997, Six *et al.*, 2002, Feng *et al.*, 2013).

**Water soluble and hot water extractable carbon (WSC and HWC respectively):**

Water soluble carbon (WSC) is the carbon extractable with cold water (20°C). It is a carbon pool available to microorganisms (Haynes 2000, Ghani *et al.*, 2003). Hot water extractable carbon (80°C) is a labile carbon pool, including microbial biomass, soluble carbohydrates and amines. It has been suggested to represent a mineralizable pool of organic matter in soils (Ghani *et al.*, 2003).

**Water soluble and hot water extractable nitrogen (WSN and HWN respectively):**

Water soluble nitrogen (WSN) is the total organic and inorganic nitrogen extractable with cold water (20°C). Hot water extractable nitrogen Jenkinson (80°C) (HWN) is related to soil microbial biomass (Sparling 1998, Curtin *et al.*, 2006) and plant nitrogen uptake (Curtin *et al.*, 2006).

**Total nitrogen (Ntot):**

Total soil nitrogen content *i.e.* organic and mineral nitrogen.

**Ratio of soil organic carbon and nitrogen (Corg\_Ntot):**

Ratio between soil total organic carbon and nitrogen contents. This is an indicator for potential nitrogen limitation of plants and other organisms. Further, it is related to the decomposability of soil organic matter and to net N mineralisation or immobilisation.

**Microbial biomass carbon and nitrogen (MBC and MBN respectively):**

Microbial biomass, mainly composed of carbon and nitrogen, is the living component of organic matter, excluding macroorganisms and roots (Jenkinson and Ladd 1981). It consists mainly of bacteria and fungi, but, if present, protozoa and algae are also included. It provides information on the transformation of soil organic matter and it is therefore important in the cycles of carbon, nitrogen and other nutrients (Dalal 1998).

**Ratio of microbial biomass carbon and microbial biomass nitrogen (MBC\_MBN):**

The MBC/MBN ratio is the ratio between microbial biomass carbon and nitrogen. This indicator provides information on the relative composition of the microbial community in bacteria and fungi.

**Potential respiration (PR)**

Potential soil respiration is a short-term measure of the rate of mineralization of organic carbon into CO<sub>2</sub> during the decomposition of organic matter by microorganisms.

**Metabolic potential of soil bacteria (MP)**

Metabolic potential of soil bacteria is a measure of the functional diversity of the soil microbial community by CLPP (Community Level Physiological Profiling). It measures the ability of soil bacteria to degrade different carbon substrates. It is a measure of functional diversity, through the estimation of the diversity of microbial functions in carbon mineralization.

**Net nitrogen mineralization (N<sub>min</sub>)**

Net nitrogen mineralization is the transformation of organic nitrogen into mineral nitrogen (NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>). It provides information on the availability of nitrogen for plants.

**Metabolic quotient (qCO<sub>2</sub>)**

The metabolic quotient is an eco-physiological quotient corresponding to the ratio between potential respiration and microbial biomass carbon (qCO<sub>2</sub>=PR/MBC). It is considered as an indicator of stress, as it is related to the maintenance energy for microbial cells (Anderson and Domsch 1985). A higher metabolic quotient indicates higher requirements for maintenance.

**Microbial quotient (q<sub>mic</sub>)**

The microbial quotient is an eco-physiological quotient corresponding to the ratio between microbial biomass carbon and total organic carbon (q<sub>mic</sub>=MBC/Corg). It is an index for carbon availability and can be used as an early indicator of changes in organic carbon. An increase or decrease in microbial carbon is assumed to be followed by a change in the same direction in total organic carbon (Anderson, 1989).

**Earthworm density (EWD)**

Earthworm density is the number of sampled earthworms divided by the sampling surface. Among the macrofauna, earthworms are considered as "key" organisms in soil functioning. They are both decomposers and soil engineers (because they modify soil structure and texture by creating galleries). Thanks to their different roles in the ecosystem, earthworms promote soil fertility (Eisenhauer 2010).

**Earthworm mass (EWM)**

Total mass of earthworms, an indicator of the potential mass of soil affected by earthworms.

**C. Data limitations**

There is some heterogeneity in the dataset, as some variables were not assessed for all samples. For example, Corg\_1wr20 and Corg\_abv20 were assessed for 172 of the 366 samples, WSC, HWC, WSN and HWN were not assessed in the first phase of the project (*i.e.* "Carbiosol 1", in 2013 and 2014) and N<sub>tot</sub> content was only assessed in 2013 and 2015. Detailed information (crop type and agricultural practice, for example) was not available for some sites. Detailed information is presented in table 3.

**Table 3: Number of samples assessed for each variable in the 4 CARBIOSOL sampling campaigns.**

Variables	CARBIO SOL 1 (2013–2014)	CARBIO SOL 2 (2015)	CARBIO SOL 3 (2016)	CARBIO SOL 4 (2016)
Corg	98	60	193	64
Corg_lwr20	37	60	101	60
Corg_abv20	37	60	101	60
WSC	0	60	143	64
HWC	0	60	144	64
WSN	0	60	144	64
HWN	0	60	144	64
Ntot	61	60	0	0
Corg_Ntot	61	60	0	0
MBC	59	60	144	64
MBN	60	60	144	64
MBC_MBN	59	60	144	64
RP	61	60	144	64
MP	57	60	144	64
Nmin	60	60	144	64
qCO2	59	60	144	64
qmic	59	60	144	64
EWD	60	60	144	64
EWM	60	60	0	0

*Corg*=total soil organic carbon content; *Corg\_lwr20*=organic carbon content in soil fraction finer than 20  $\mu\text{m}$ ; *Corg\_abv20*=organic carbon content in soil fraction coarser than 20  $\mu\text{m}$ ; *WSC*=water soluble carbon; *HWC*=hot water extractable carbon; *WSN*=water soluble nitrogen; *HWN*=hot water extractable nitrogen; *Ntot*=total soil nitrogen content; *Corg\_Ntot*=ratio between soil organic carbon and nitrogen contents; *MBC*=microbial biomass carbon; *MBN*=microbial biomass nitrogen; *MBC\_MBN*=ratio between microbial biomass carbon and microbial biomass nitrogen; *RP*=respiration potential; *MP*=metabolic potential of soil bacteria; *Nmin*=net nitrogen mineralisation; *qCO2*=metabolic quotient= $RP/MBC$ ; *qmic*=microbial quotient= $MBC/Corg$ ; *EWD*=earthworm density; *EWM*=earthworm mass.

## **Class V. Data set references**

Data from analyses and not from literature.

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