

Table S1. Compilation of data from our long-term experiment comparing no-tillage (NT) and conventional tillage (CT) under crop rotation R2 (black oat/soybean/black oat + common vetch/maize/oilseed radish /wheat/soybean).

Attribute	Unit	Age (years) or period of experiment	Soil depth (cm)	NT	CT
Carbon and nitrogen inputs to the soil, soil stocks and nitrogen balance					
¹ Average annual C input (aboveground+30% root contrib.)	Mg ha ⁻¹ year ⁻¹	Jun 1999–Jun 2001	-	4.1	3.3
² Average annual aboveground C input	Mg C ha ⁻¹ year ⁻¹	1985–2007	-	2.9/1.6/2.5/4.7/2.9/1.5	2.5/1.4/2.1/3.8/2.6/1.5
Soybean/wheat/oat/maize/oat+vetch/oilseed radish					
² Average annual aboveground C input	Mg C ha ⁻¹ year ⁻¹	1985–2007	-	6.1	5.3
³ Average annual N input by crops + mineral fertilization	Mg ha ⁻¹ year ⁻¹	1985–2004	-	0.079	0.076
⁴ Average annual above ground N input (cover crops)	Kg ha ⁻¹	1998–2000	-	233	224
³ Average C/N of residues (incl. mineral N fertilization)	-	1985–2004	-	78.4	70.3
² SOC stock	Mg ha ⁻¹	22	0–5/5–15/15–30	15.7/22.9/34.4	10.5/25.4/30.4
² SOC stock	Mg ha ⁻¹	22	30–45/45–60/60–90	24.3/19.5/26.5	22.7/19.2/26.0
⁵ Total N stock	Mg ha ⁻¹	22	0–30/0–100	6.5/13.8	5.1/12.1
Nitrogen balance = (maize+wheat mineral nitrogen fertilization) – (maize+wheat+soybean grain exportation)	Kg ha ⁻¹	22	-	-2639	-2017
Soil organic matter protection mechanisms					
³ Particulate organic C stock	Mg ha ⁻¹	19	0–5/5–10/10–20/20–30	3.2/0.7/0.5/0.3	1.9/1.1/0.9/0.3
³ Mineral associated organic C stock	Mg ha ⁻¹	19	0–5/5–10/10–20/20–30	14.6/11.8/20.9/19.4	12.9/11.8/20.5/18.5
³ Particulate total N	Mg ha ⁻¹	19	0–5/5–10/10–20/20–30	0.23/0.06/0.05/0.03	0.12/0.09/0.08/0.02
³ Total N associated to minerals	Mg ha ⁻¹	19	0–5/5–10/10–20/20–30	1.3/0.9/1.6/1.5	1.0/0.9/1.6/1.4
³ Particulate organic matter fraction C/N ratio	-	19	0–5/5–10/10–20/20–30	14.2/12.2/8.5/10.3	16.0/13.2/11.5/13.9
³ Mineral associated organic matter fraction C/N ratio	-	19	0–5/5–10/10–20/20–30	11.8/12.6/13.0/13.0	12.6/12.5/12.7/13.3
¹ C management index	-	19	0–30	158	130
² C content in aggregate classes <53/53–250/250–2000/ >2000 µm	g 100 g ⁻¹ fraction	22	0–5	4.0/3.7/3.1/3.6	2.4/2.7/2.3/2.5
⁶ C groups in humic acids in aggregates (150–250 µm) Carboxyl/Aromatic/O-alkyl/Alkyl	%	27	0–5	7.6/50.6/21.3/20.5	1.4/35.2/31.2/23.2
⁶ Humic acids hydrophilic/hydrophobic C	%	27	0–5	59.8/40.2	71.3/28.7
Physical attributes					
⁷ Aggregate classes <53/53–250/250–2000/ >2000 µm	g 100 g ⁻¹ soil	22	0–5	0.7/2.2/10.9/86.2	0.8/2.4//12.7/84.2
⁸ Geometric mean diameter of aggregates	mm	7	0–5	2.23	1.38
² Soil bulk density	Mg m ⁻³	22	0–5/5–10/10–15/15–20	1.1/1.3/1.4/1.4	1.2/1.2/1.2/1.3

Soil microbiota and enzyme activity					
⁹ Microbial biomass	nmol g ⁻¹ soil	32	0–5/5–10/10–30	44.2/23.0/12.4	28.2/26.3/18.8
⁹ Total bacteria	nmol g ⁻¹ soil	32	0–5/5–10/10–30	16.5/10.0/5.8	11.9/11.3/8.4
⁹ Total fungi	nmol g ⁻¹ soil	32	0–5/5–10/10–30	9.6/5.5/2.3	6.4/5.9/3.9
⁹ Actinomycetes	nmol g ⁻¹ soil	32	0–5/5–10/10–30	1.6/1.2/0.7	1.2/1.2/0.9
⁹ Arbuscular mycorrhizal fungi	nmol g ⁻¹ soil	32	0–5/5–10/10–30	6.3/4.0/1.8	4.3/4.1/3.0
⁹ B-glucosidase	nmol ⁻¹ hr ⁻¹ g ⁻¹ soil	32	0–5/5–10/10–30	301.0/95.7/63.6	76.1/65.4/48.7
⁹ N-acetyl-glucosaminidase	nmol ⁻¹ hr ⁻¹ g ⁻¹ soil	32	0–5/5–10/10–30	139.7/141.5/133.5	62.2/67.0/96.4
CO ₂ -C flux, residues decomposition and C sequestration rate					
¹⁰ Annual CO ₂ -C efflux	Mg ha ⁻¹	1999–2001	-	9.5	8.9
¹⁰ t _{1/2} black oat+vetch residues	Day	Nov 1998–Apr 1999	-	124	54
¹⁰ t _{1/2} wheat residues	Day	Nov 1999–Apr 2000	-	177	82
¹¹ Average CO ₂ -C emission	Kg ha ⁻¹ day ⁻¹	Sep–Oct 2007	-	28.0	48.1
¹² Average CO ₂ -C emission	Kg ha ⁻¹ day ⁻¹	May – Sep 2010	-	24.4	-
² Average apparent C sequestration rate	Mg ha ⁻¹ year ⁻¹	1985–2007	0–30/0–60/0–90	0.38/0.48/0.52	0.07/0.08/0.09
Crop yields					
Average yield (soybean/maize)	Kg ha ⁻¹	1985–2007	-	3064/6082	2461/5089

¹ Campos et al. [39], ² Nicoloso [38], ³ Campos [60], ⁴ Ferreira et al. [11], ⁵ Nicoloso et al. [41], ⁶ Arachchige et al. [27], ⁷ Fiorin et al. [45], ⁸ Campos et al. [37],
² Pires et al. [44], ¹⁰ Campos et al. [40], ¹¹ Pes et al. [46], ¹² Bortolotto et al. [61].

- ³ 11. Ferreira, A.O.; Amado, T.J.C.; Nicoloso, R.S.; Sá, J.C.M.; Fiorin, J.E.; Hansel, D.S.S.; Menefee, D. Soil carbon stratification affected by long-term tillage and cropping systems
⁴ in southern Brazil. *Soil Tillage Res.* **2013**, *133*, 65–74. doi: 10.1016/j.still.2013.05.011
- ⁵ 27. Arachchige, P.S.P.; Hettiarachchi, G.M.; Rice, C.W.; Dynes, J.J.; Maumann, L.; Wang, J.; Karunakaran, C.; Kilcoyne, A.L.D.; Attanayake, C.P.; Amado, T.J.C.; Fiorin, J.E.
⁶ Sub-micron level investigation reveals the inaccessibility of stabilized carbon in soil microaggregates. *Sci. Rep.* **2018**, *8*, 1–13. doi: 10.1038/s41598-018-34981-9
- ⁷ 37. Campos, B.C.; Reinert, D.J.; Nicolidi, R.; Ruedell, J.; Petrere, C. Soil structural stability of a Dar-Red Latosol after seven years under crop rotation and management
⁸ systems. *Rev. Bras. Ciênc. Solo* **1995**, *19*, 121–126.
- ⁹ 38. Nicoloso, R.S. Soil organic carbon stocks and stabilization mechanisms on temperate and sub-tropical climate agroecosystems. PhD Thesis, Universidade Federal de Santa
¹⁰ Maria, Rio Grande do Sul, Brazil. 21 July 2009.
- ¹¹ 39. Campos, B.C.; Carneiro, T.J.C.; Bayer, C.; Nicoloso, R.D.S.; Fiorin, J.E. Carbon stock and its compartments in a subtropical Oxisol under long-term tillage and crop rotation
¹² systems. *Rev. Bras. Cienc. Solo* **2011**, *35*, 805–817. doi: 10.1590/S0100-06832011000300016
- ¹³ 40. Campos, B.C.; Amado, T.J.C.; Tornquist, C.G.; Nicoloso, R. S.; Fiorin, J. E. Long-term C-CO₂ emissions and carbon crop residue mineralization in an Oxisol under different
¹⁴ tillage and crop rotation systems. *Rev. Bras. Ciênc. Solo* **2011**, *35*, 819–832. doi: 10.1590/S0100-06832011000300017
- ¹⁵ 41. Nicoloso, R.S.; Amado, T.J.C.; Rice, C.W.; Pires, C.A.B.; Fiorin, J.E. A rotação de culturas aumenta os estoques de carbono e nitrogênio no solo sob sistema plantio direto.
¹⁶ In *Resultados comparativos de 32 anos dos Sistemas Plantio Direto e Convencional*, 1st ed.; Fiorin, J.E., Ruedell, J., Fernandes, A.M.F., Eds.; Sescoop/RS: Rio Grande do Sul,
¹⁷ Brazil, 2019; Volume 1, pp. 143–155. ISBN 978-85-63500-43-4

- 18 44. Pires, C.A.B.; Amado, T.J.C.; Reimche, G.; Schwalbert, R.; Sarto, M.V.M.; Nicoloso, R.S.; Fiorin, J.E.; Rice, C.W. Diversified crop rotation with no-till changes microbial
19 distribution with depth and enhances activity in a subtropical Oxisol. *Eur. J. Soil Sci.* **2020**. doi: 10.1111/ejss.12981
- 20 45. Fiorin, J.E.; Ruedell, J.; Fernandes, A.M.F. Efeito dos sistemas de manejo de solo e da rotação de culturas sobre o rendimento de grãos de milho, soja e trigo. In *Resultados*
21 *comparativos de 32 anos dos Sistemas Plantio Direto e Convencional*, 1st ed.; Fiorin, J.E., Ruedell, J., Fernandes, A.M.F., Eds.; Sescoop/RS: Rio Grande do Sul, Brazil, 2019;
22 Volume 1, pp. 65–82. ISBN 978-85-63500-43-4
- 23 46. Pes, L.Z.; Amado, T.J.C.; La Scala, N.; Bayer, C.; Fiorin, J.E. The primary sources of carbon loss during the crop-establishment period in a subtropical Oxisol under
24 contrasting tillage systems. *Soil Tillage Res.* **2011**, *117*, 163–171. doi: 10.1016/j.still.2011.10.002
- 25 60. Campos, B.C. Carbon dynamics on a Rhodic Hapludox on soil tillage and crop systems. PhD Thesis, Universidade Federal de Santa Maria, Rio Grande do Sul, Brazil. 27
26 April 2006.
- 27 61. Bortolotto, R.P.; Amado, T.J.C.; Nora, D.D.; Keller, C.; Roberti, D.; Fiorin, J.E.; Reichardt, K.; Zamberlan, J. F.; Pasini, M.P.B.; Nicoloso, R.S. Soil carbon dioxide flux in a
28 no-tillage winter system. *Afri. J. Agr. Res.* **2015**, *10*, 450–457. doi: 10.5897/AJAR2014.9399
- 29

Table S2. F-statistics (F), degrees of freedom (df) and probability values (*p*) for effects of tillage system (TS), cover crops (CC) and their interaction (TS x CC) on dry biomass (a), carbon (C) input by CC (b), nitrogen input by CC (c), daily average CO₂-C emission from the 28 measuring days (d), total accumulated CO₂-C emission during the 124 days (e), C in the CC biomass remaining in the litter-bags after 124 days (f), CC residues half-life time (g), and apparent C balance (h).

Response variables	TS		CC		TS x CC	
	F _{df}	<i>p</i>	F _{df}	<i>p</i>	F _{df}	<i>p</i>
Dry biomass ^(a)	0.00 _{1,18}	0.955	5.0 _{2,18}	0.0307	0.65 _{2,18}	0.544
C input ^(b)	0.01 _{1,18}	0.937	10.8 _{2,18}	<0.0001	1.17 _{2,18}	0.333
N input ^(c)	0.12 _{1,18}	0.739	610.3 _{2,18}	<0.0001	0.31 _{2,18}	0.738
CO ₂ -C emission (28 days) ^(d)	404 _{2,36}	<0.0001	367 _{3,36}	<0.0001	16.5 _{6,36}	<0.0001
CO ₂ -C emission (124 days) ^(e)	123 _{1,15}	<0.0001	16.1 _{2,15}	0.0002	8.09 _{2,15}	0.0041
Remaining C (124 days) ^(f)	189 _{1,15}	<0.0001	106 _{2,15}	<0.0001	4.35 _{2,15}	0.0323
CC half-life time ^(g)	313 _{1,18}	<0.0001	43.7 _{2,18}	<0.0001	18.1 _{2,18}	<0.0001
Apparent C balance ^(h)	68 _{1,15}	<0.0001	5.01 _{2,15}	<0.0216	7.43 _{2,15}	<0.0057

Table S3. Average soil temperature, moisture and water-filled space porosity (WFSP) as affected by soil tillage system: conventional tillage (CT), minimum tillage (MT) and no-tillage (NT) and cover crops: no cover crop (ncc), oat, oat+vetch and vetch. Values are means of 28 measuring days performed within the whole experimental period (124 days).

Tillage system	Cover crop	Soil temperature °C	Soil moisture $\text{m}^3 \text{ m}^{-3}$	Soil WFSP %
CT	ncc	28.9	0.15	44.9
	oat	28.4	0.16	45.6
	oat+vetch	28.9	0.16	45.1
	vetch	28.2	0.17	45.0
MT	ncc	29.0	0.19	44.8
	oat	27.8	0.22	45.9
	oat+vetch	27.7	0.21	45.4
	vetch	27.9	0.21	45.0
NT	ncc	29.1	0.20	47.1
	oat	27.0	0.20	47.7
	oat+vetch	27.3	0.21	47.0
	vetch	27.5	0.20	47.6

Table S4. Relationship between CO₂ emission and soil temperature and moisture.

Tillage system	Cover crop	Period (weeks)	Soil moisture		Soil temperature	
			Regression equation	R ²	Regression equation	R ²
CT	ncc	1st	y = -0.0076x + 0.3601	0.48*	y = -0.3106x + 38.083	0.41*
		2nd	y = 0.0099x - 0.0994	0.15*	-	ns
		3rd–6th	-	ns	-	ns
		7th–18th	-	ns	-	ns
	oat	1st	-	ns	-	ns
		2nd	y = 0.0058x - 0.0839	0.81**	-	ns
		3rd–6th	y = 0.0043x - 0.0338	0.20*	-	ns
		7th–18th	-	ns	-	ns
MT	oat+vetch	1st	-	ns	-	ns
		2nd	y = 0.0018x + 0.0791	0.25*	y = 0.2251x + 16.29	0.35*
		3rd–6th	-	ns	-	ns
		7th–18th	y = -0.0031x + 0.3294	0.28*	y = 0.0821x + 20.854	0.16*
	vetch	1st	-	ns	-	ns
		2nd	y = 0.0017x + 0.0959	0.23*	y = 0.1475x + 18.617	0.24*
		3rd–6th	y = 0.0061x - 0.1236	0.66**	y = -0.324x + 48.229	0.16*
		7th–18th	-	ns	-	ns
NT	ncc	1st	y = 0.0147x - 0.0919	0.81**	y = -0.299x + 35.504	0.51*
		2nd	-	ns	y = 0.905x + 11.4	0.70***
		3rd–6th	y = -0.029x + 0.7385	0.95**	y = 1.1873x + 9.0283	0.60**
		7th–18th	y = -0.0059x + 0.3944	0.30*	y = 0.2199x + 18.241	0.34**
	oat	1st	-	ns	-	ns
		2nd	y = 0.0022x + 0.1639	0.49**	y = 0.1673x + 17.973	0.38**
		3rd–6th	y = 0.0171x - 0.3121	0.38*	y = -1.486x + 74.388	0.58*
		7th–18th	y = -0.0066x + 0.4372	0.31*	-	ns
RT	oat+vetch	1st	-	ns	-	ns
		2nd	y = 0.0014x + 0.1834	0.40*	y = 0.048x + 23.494	0.23**
		3rd–6th	y = 0.0119x - 0.2451	0.58**	y = -0.871x + 61.528	0.87***
		7th–18th	-	ns	y = 0.1293x + 19.948	0.20*
	vetch	1st	-	ns	-	ns

		2nd	$y = 0.0025x + 0.055$	0.79**	-	ns
		3rd–6th	$y = 0.0065x - 0.0928$	0.51**	$y = -0.1879x + 41.21$	0.22*
		7th–18th	$y = -0.003x + 0.3578$	0.22*	$y = 0.0827x + 21.798$	0.13*
NT	ncc	1st	$y = 0.0069x + 0.0882$	0.36**	-	ns
		2nd	$y = 0.0069x + 0.0924$	0.34*	$y = 0.2693x + 22.498$	0.16*
		3rd–6th	$y = 0.0063x + 0.0391$	0.24*	$y = -0.560x + 42.693$	0.21*
		7th–18th	-	ns	-	ns
	oat	1st	$y = 0.0036x + 0.1304$	0.87***	-	ns
		2nd	-	ns	-	ns
		3rd–6th	-	ns	$y = -1.837x + 60.359$	0.82***
		7th–18th	-	ns	-	ns
	oat+vetch	1st	$y = 0.0017x + 0.2113$	0.26*	$y = -0.053x + 28.737$	0.49**
		2nd	$y = 0.0027x + 0.1292$	0.50*	-	ns
		3rd–6th	$y = 0.009x - 0.0127$	0.33*	$y = -0.484x + 38.521$	0.55**
		7th–18th	-	ns	$y = -0.200x + 34.569$	0.30*
	vetch	1st	$y = 0.0011x + 0.2347$	0.63***	$y = -0.029x + 27.763$	0.15*
		2nd	$y = 0.0017x + 0.1572$	0.21*	-	ns
		3rd–6th	$y = 0.0106x - 0.0635$	0.51**	$y = -0.412x + 38.571$	0.54**
		7th–18th	-	ns	-	ns

Tillage system: conventional tillage (CT), minimum tillage (MT) and no-tillage (NT). Cover crops: no cover crop (ncc), oat, oat+vetch and vetch. Levels of significance: * p < 0.05; ** p < 0.01; *** p < 0.001; ns = non-significant.