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Comparing modified substrate-induced respiration with selective inhibition (SIRIN) and N_2O isotope approaches to estimate fungal contribution to denitrification in three arable soils under anoxic conditions

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Table S1: Important terms used in the present study and descriptions of terms with presenting the associated sections.

Term	Description	Eq.	Section
NO_3^- NO_2^- NO N_2O N_2 KNO_3 NH_4^+ CO_2 C_2H_2 O	Nitrate: electron acceptor for denitrification Nitrite: electron acceptor for denitrification Nitrogen monoxide: intermediate of denitrification Nitrous oxide: intermediate or product of denitrification Dinitrogen: end product of denitrification Potassium nitrate: electron acceptor for denitrification Ammonia Carbon dioxide: product of respiration Acetylene used to block the N_2O reductase oxygen	/	1, 2
Nos	N_2O reductase	/	1
$\delta^{15}N^{bulk}_{N2O}$	$\delta^{15}N$ values of produced N_2O	/	1
$\delta^{15}N_{NOx}$	$\delta^{15}N^{bulk}$ values of N_2O precursors NO_3^- or NO_2^-	/	2.1
SP_{N2O}	^{15}N site preference of N_2O; i.e. difference between $\delta^{15}N$ of the central and terminal N-position of the asymmetric N_2O molecule (Toyoda and Yoshida, 1999).	/	1, 2.3, 2.5
$\delta^{18}O_{N2O}$	$\delta^{18}O$ values of produced N_2O	/	1
$\delta^{18}O_{NOx}$	$\delta^{18}O$ values of N_2O precursors NO_3^- or NO_2^-	/	1
$\delta^{18}O_{H2O}$	$\delta^{18}O$ values of water (H_2O)	/	1, 2.5.2
Soil 1.1 *Soil 1.2* *Soil 2* *Soil 3*	loamy sand sampled in June 2011 loamy sand sampled in December 2012 sand sampled in January 2013 silt loam sampled in December 2012	/	2.1; Table 1
F:B	Respiratory fungal-to-bacterial ratio analysed by SIRIN method (Anderson and Domsch, 1973, 1975)	/	1, 2.2; Table 1
SIR	Substrate-induced respiration	/	2.2.1; Table 1
c_{opt}*(cycloheximide)*, c_{opt}*(streptomycin))*	optimal concentration for inhibition of fungal respiration		2.1
SIRIN treatment A treatment B treatment C treatment B	Substrate-induced respiration with selective inhibition (Anderson and Domsch, 1973, 1975) without addition of inhibitor, but amended with glucose with addition of inhibitor for bacterial growth (streptomycin) and glucose with addition of inhibitor for fungal growth (cycloheximide) and glucose with addition of bot inhibitors (streptomycin, cycloheximide) and glucose	1, 2, 3	1, 2.2.1, 2.2.2, 2.4
f_{FDmi}	fungal contribution to N_2O production during denitrification with microbial inhibition	3	Table 5
Variant *traced* Variant $+C_2H_2$ Variant $-C_2H_2$	^{15}N tracer technique was used to estimate the effect of N_2O reduction on N_2O produced Natural isotopic conditions and C_2H_2 addition to the headspace (10 kPa) to block N_2O reduction Natural isotopic conditions and no C_2H_2 addition to the headspace	/	1; 2.2.2; Figure 1
WFPS	Water filled pore space	/	2.2
GC	Gas chromatography	/	2.3
$c(N_2O)$, $c(CO_2)$	N_2O and CO_2 concentrations analysed by GC	/	2.3, Figure 1
IRMS	Isotope ratio mass spectrometry	/	2.5

IEM	the isotope endmember mixing approach proposed by Ostrom et al. (2010)	/	1, 2.5.1
SP_{prod}	SP_{N2O} values of N_2O produced in soil	4	1, 2.5.1
f_{FD}	Fraction of fungi contributing to N_2O production during denitrification	4	2.5.1
f_{BD}	Fraction of bacteria contributing to N_2O production during denitrification	4	2.5.1
SP_{FD}	SP_{N2O} values produced by fungi contributing to N_2O production during denitrification	4	2.5.1
SP_{BD}	SP_{N2O} values produced by bacteria contributing to N_2O production during denitrification	4	2.5.1
f_{FD_SP}	SP_{N2O} values produced by fungi calculated with IEM using results of variant *+C_2H_2;* assuming SP_{N2O} values of N_2O produced by bacteria were 3.7 ‰ (resulting in negative fraction and therefore set to zero) or -7.5 ‰. Using the minimum and maximum SP_{N2O} values known for bacteria resulted in a f_{FD_SP} range.	4	2.5.1, Table 5
f_{FD_SPpot}	Maximum potential fungal fraction of N_2O production calculated by with IEM for all treatments of variant -C_2H_2 assuming SP_{N2O} values of N_2O produced by bacterial denitrification or nitrifier denitrification were between 3.7 and -10.7 ‰ (Frame and Casciotti, 2010; Yu et al., 2020) or produced by fungal denitrification or nitrification were between 16 and 37 ‰ (Sutka et al., 2008; Decock and Six, 2013; Rohe et al., 2014a; Maeda et al., 2015; Rohe et al., 2017). Here, the effect of potential partial reduction of N_2O could not be included.	4	2.5.1, Table 5
SP/δ^{18}O Map	isotope mapping approach was further developed (SP/δ^{18}O Map) using $\delta^{18}O_{N2O}$ and SP_{N2O} values of N_2O and δ^{18}O values of precursors (Lewicka-Szczebak et al., 2017; Lewicka-Szczebak et al., 2020)	/	1, 2.5.2
f_{FD_MAP}	f_{FD} contributing to N_2O production from denitrification in soil samples estimated with the SP/δ^{18}O Map	/	2.5.2, Table 4, Table 5
r_{MAP}	N_2O product ratio [$N_2O/(N_2+N_2O)$] estimated with the SP/δ^{18}O Map	/	2.5.2
r_{15N}	N_2O product ratio [$N_2O/(N_2+N_2O)$] derived from variant *traced*	5	2.5.3
$^{15}N_{N2O}$, $^{15}N_{N2}$	^{15}N-labeling of N_2O or N_2 produced	5	2.5.3
r_{C2H2}	N_2O product ratio [$N_2O/(N_2+N_2O)$] calculated from N_2O production rates of variants -C_2H_2 and +C_2H_2	6	2.5.3
N_2O_{-C2H2} N_2O_{+C2H2}	N_2O produced in variants -C_2H_2 and +C_2H_2, respectively	6	2.5.3
SP_{N2O-r}	^{15}N site preference values of produced N_2O, i.e. without its reduction to N_2O (SP_{prod}), of variant -C_2H_2	7	2.5.3
ηr	Net isotope effect of N_2O reduction	7	2.5.3
$\delta 0$	isotopic values of N_2O produced without N_2O reduction effects of variant +C_2H_2	/	2.5.3
f_{FD_SPcalc}	From variant -C_2H_2, SP_{N2O} values of N_2O produced by bacteria was 3.7 (resulting in negative fraction and therefore set to zero) or -7.5 ‰ and using reduction correction with η_r=-6 ‰ to calculate SP_{prod} values (Senbayram et al., 2018; Yu et al., 2020). Using the minimum and maximum SP_{N2O} values known for bacteria resulted in a f_{FD_SP} range.	7	2.5.3, Table 5
a_p	calculate the fraction of N_2 and N_2O originating from the ^{15}N-labelled N pool as well as the ^{15}N enrichment of that N pool	/	4.4
$^{15}N_{N2O\_exp}$	expected ^{15}N enrichment in N_2O produced assuming that denitrification is the only process producing N_2O in the incubation experiment	8	2.6
N_{soil}, N_{fert}, N^{bulk}	amount of N [mg] in unfertilized soil samples	8	2.6

$^{15}N_{nat}$, $^{15}N_{fert}$	^{15}N enrichment under natural conditions (0.3663 at%) and in fertilizer (50 at%), respectively	8	2.6

Determining optimal concentrations for SIR and SIRIN

As described in the Material and Methods section, optimal concentrations of glucose or inhibitors streptomycin and cycloheximide were determined by SIR or SIRIN method using an automated incubation system using an "Ultragas 3" CO_2 analyser (WösthoffCo., Bochum) with continuous gas flow and analysed with the software "SIR-SBA 4.00" (Heinemeyer, copyright MasCo Analytik, Hildesheim, Germany) (Anderson and Domsch, 1973, 1975, 1978). This program enabled to analyse respiration curves for biomass and F:B ratio in soil. However, as data were generated by this software of the incubation system raw data could not be exported and it is thus not possible to represent all tested concentrations and replicates for one soil in one figure. Therefore, results for one representative replicate with glucose concentrations between 0.75 and 2 mg g^{-1} soil as an example is presented for *Soils 1 to 3* (Figure S1). Additionally, one representative respiration curve of pre-experiments using the SIRIN approach is represented as an example for each with optimum concentrations of streptomycin and cyclohexmide (Figure S2).

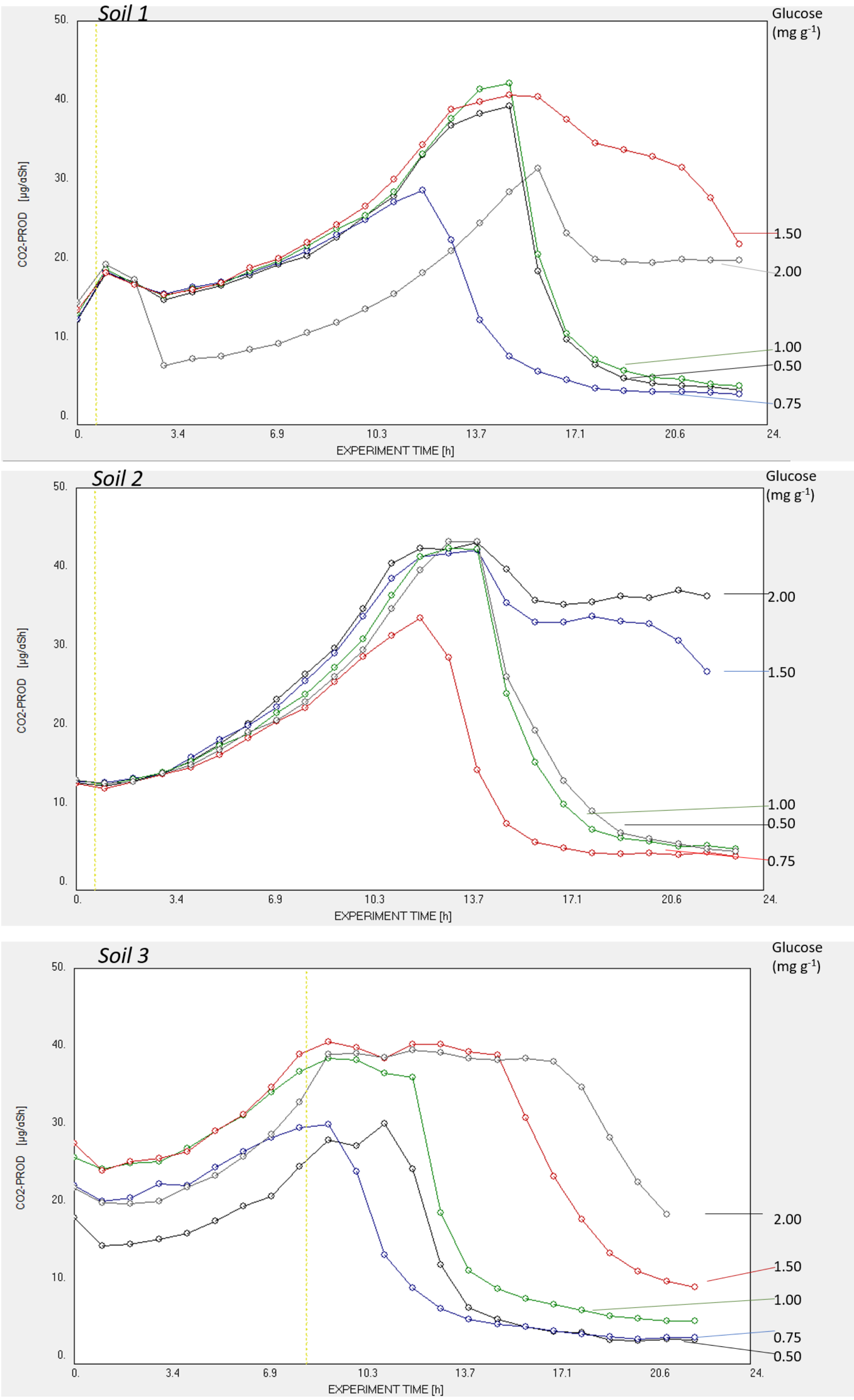

Figure 1: Respiration curves of pre-experiments derived from data analysis using the computer program "SIR-SBA 4.00" (Heinemeyer, copyright MasCo Analytik, Hildesheim, Germany) for *Soils 1 -3*. Here results for experiments with glucose concentrations between 0.5 and 2 mg g^{-1} are presented as examples for one replicate each.

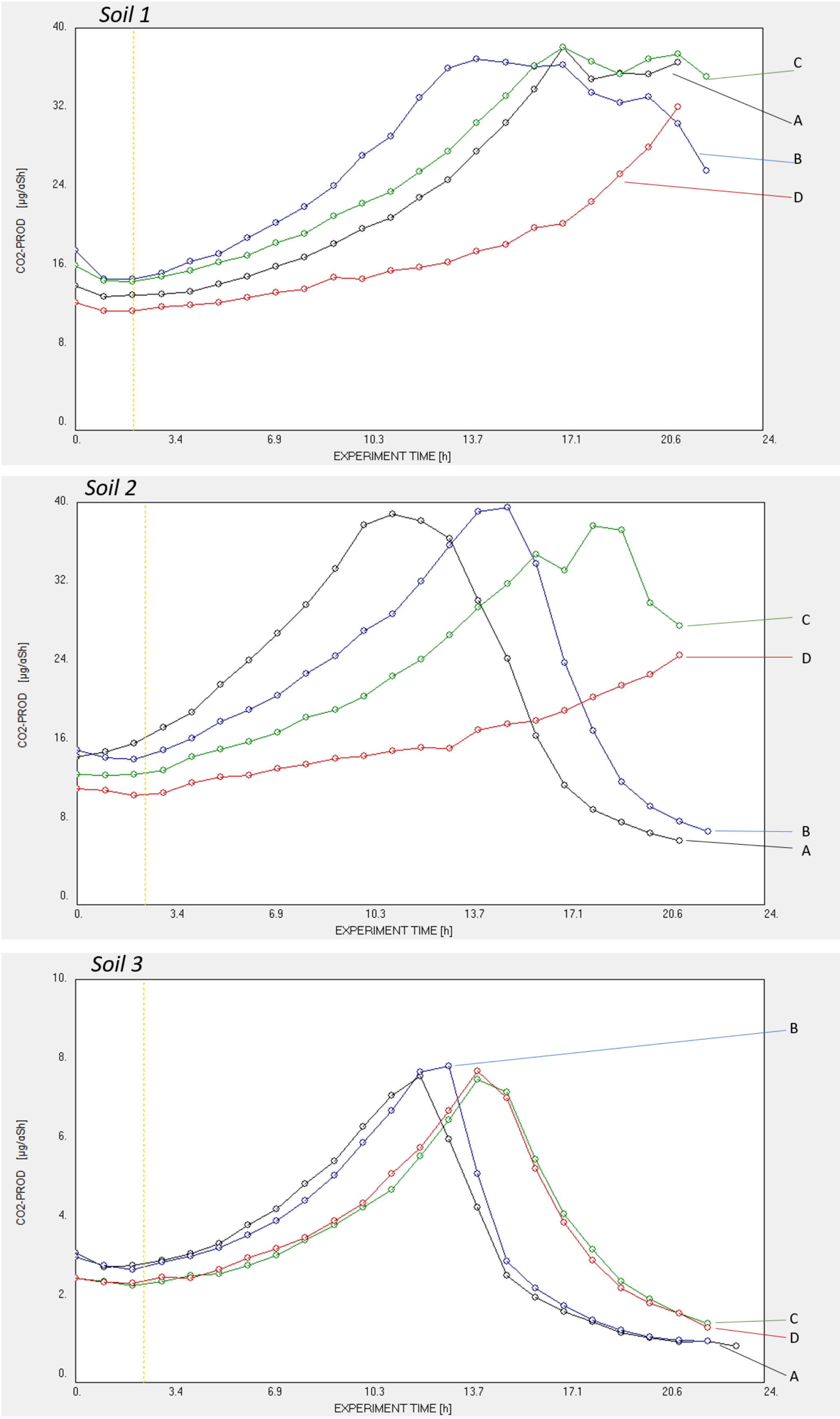

Figure S2: Respiration curves of the pre-experiment for SIRIN approach derived from data analysis using the computer program "SIR-SBA 4.00" (Heinemeyer, copyright MasCo Analytik, Hildesheim, Germany) with optimum inhibitor concentrations. The examples represent treatment A without growth inhibition, treatment B with 1.0 mg g^{-1} dw soil

streptomycin, treatment C with 0.75 mg g^{-1} dw soil cycloheximide and D with both inhibitors for experiments with *Soil 1-3*. Results show curves as an example for one replicate each.

Table S2: SP values of produced N_2O, i.e. without its reduction to N_2, of variant –C_2H_2 (SP_{prod}) calculated by the Rayleigh-type model according to Lewicka-Szczebak et al. (2017) and Senbayram et al. (2018) (Eq. 7) using the isotope effect of N_2O reduction from the literature (-6‰) (Yu et al., 2020) and the r_{15N}.

Experiment	Treatment/variant	SP_{prod}.
Soil 1.1 (Loamy sand, summer 2011)	A / -C_2H_2	2.71
	B / -C_2H_2	-1.80
	C / -C_2H_2	2.40
	D / -C_2H_2	-0.71
Soil 1.2 (Loamy sand, winter 2012)	A / -C_2H_2	0.91
	B / -C_2H_2	0.37
	C / -C_2H_2	1.06
	D / -C_2H_2	-0.03
Soil 2 (Sand, winter 2012)	A / -C_2H_2	-1.00
	B / -C_2H_2	-1.64
	C / -C_2H_2	-1.40
	D / -C_2H_2	-1.03
Soil 3 (Silt loam, winter 2013)	A / -C_2H_2	0.02
	B / -C_2H_2	-0.62
	C / -C_2H_2	-0.89
	D / -C_2H_2	-1.43

References:

Anderson, J. P. E., and Domsch, K. H.: Quantification of bacterial and fungal contributions to soil respiration, Archiv Fur Mikrobiologie, 93, 113-127, doi:10.1007/BF00424942, 1973.

Anderson, J. P. E., and Domsch, K. H.: Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soil, Canadian Journal of Microbiology, 21, 314-322, doi: 10.1139/m75-045, 1975.

Anderson, J. P. E., and Domsch, K. H.: Physiological method for quantitative measurement of microbial biomass in soils, Soil Biol. Biochem., 10, 215-221, doi:10.1016/0038-0717(78)90099-8, 1978.

Lewicka-Szczebak, D., Augustin, J., Giesemann, A., and Well, R.: Quantifying N_2O reduction to N_2 based on N_2O isotopocules – validation with independent methods (helium incubation and ^{15}N gas flux method), Biogeosciences, 14, 711-732, doi: 10.5194/bg-14-711-2017, 2017.

Lewicka-Szczebak, D., Lewicki, M. P., and Well, R.: N_2O isotope approaches for source partitioning of N_2O production and estimation of N_2O reduction – validation with the ^{15}N gas-flux method in laboratory and field studies, Biogeosciences, 17, 5513-5537, 10.5194/bg-17-5513-2020, 2020.

Ostrom, N. E., Sutka, R., Ostrom, P. H., Grandy, A. S., Huizinga, K. M., Gandhi, H., von Fischer, J. C., and Robertson, G. P.: Isotopologue data reveal bacterial denitrification as the primary source of N_2O during a high flux event following cultivation of a native temperate grassland, Soil Biol. Biochem., 42, 499-506, doi: 10.1016/j.soilbio.2009.12.003, 2010.

Senbayram, M., Well, R., Bol, R., Chadwick, D. R., Jones, D. L., and Wu, D.: Interaction of straw amendment and soil NO_3^- content controls fungal denitrification and denitrification product stoichiometry in a sandy soil, Soil Biol. Biochem., 126, 204-212, doi: 10.1016/j.soilbio.2018.09.005, 2018.

Toyoda, S., and Yoshida, N.: Determination of nitrogen isotopomers of nitrous oxide on a modified isotope ratio mass spectrometer, Anal. Chem., 71, 4711-4718, 10.1021/ac9904563, 1999.

Yu, L., Harris, E., Lewicka-Szczebak, D., Barthel, M., Blomberg, M. R. A., Harris, S. J., Johnson, M. S., Lehmann, M. F., Liisberg, J., Müller, C., Ostrom, N. E., Six, J., Toyoda, S., Yoshida, N., and Mohn, J.: What can we learn from N_2O isotope data? - Analytics, processes and modelling, Rapid Commun Mass Spectrom, doi: 10.1002/rcm.8858, 2020.