Comprehensive increase in CO_2 release by drying-rewetting cycles among Japanese forests and pastureland soils and exploring predictors of increasing magnitude

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Abstract. It is still difficult to precisely quantify and predict the effects of drying-rewetting cycles (DWCs) on soil carbon dioxide (CO_2) release due to the paucity of studies using constant moisture conditions equivalent to the mean water content during DWC incubation. The present study was performed to evaluate overall trends in the effects of DWCs on CO_2 release and to explore environmental and soil predictors for variations in the effect size in 10 Japanese forests and pastureland soils variously affected by volcanic ash during their pedogenesis. Over an 84-day incubation period including three DWCs, CO_2 release was 1.3- to 3.7-fold greater than under continuous constant moisture conditions ($p < 0.05$) with the same mean water content as in the DWC incubations. Analysis of the relations between this increasing magnitude of CO_2 release by DWCs (IF_{CO2}) and various environmental and soil properties revealed significant positive correlations between IF_{CO2} and soil ~~organometal~~ organo-metal complex contents ($p < 0.05$), especially pyrophosphate extractable aluminum (Alp) content ($r = 0.74$). Molar ratios of soil total carbon (C) and pyrophosphate-extractable C (Cp) to Alp contents and soil carbon content-specific CO_2 release rate under continuous constant moisture conditions (qCO$_2$_soc) were also correlated with IF_{CO2} ($p < 0.05$). The co-variations among Alp, total C, and Cp to Alp molar ratios and qCO$_2$_soc suggested Alp as the primary predictor of IF_{CO2}. ~~Whereas~~ Additionally, soil microbial biomass C and nitrogen (N) levels were significantly lower in DWCs than under continuous constant moisture conditions, whereas there was no significant relation between the microbial biomass decrease and IF_{CO2}. The present study showed a comprehensive increase in soil CO_2 release by DWC in Japanese forests and pastureland soils, suggesting that Alp is a predictor of the effect size likely due to vulnerability of organo-Al complexes to DWC.

1   Introduction

There is accumulating evidence of climate change-induced alterations in global water cycling (IPCC, 2021; Allan et al., 2020; Dai, 2012; Donat et al., 2016; Pfleiderer et al., 2019). Of the consequent water regime changes, decreasing precipitation frequency (e.g., number of rainy days) and increasing intensity (e.g., number of heavy rainy days) are becoming more frequent (Dai, 2012; Donat et al., 2016), although decadal trends in annual precipitation levels are not significant over global scales (IPCC, 2021). For example, the annual number of precipitation days in Japan has decreased by 15% during the past 120 years, whereas the annual number of heavy precipitation days (more than 100 mm in a day) has increased by 26% (Ministry of Education, Science, and Technology (MEXT) and Japanese Meteorological Agency (JMA), 2020). There is a non-significant trend in annual precipitation level during the same period (MEXT and JAM, 2020). This changing pattern in precipitation is often observed in the temperate region of the northern hemisphere (IPCC, 2021) and is related to increased fluctuation of soil water environments, especially DWCs, and consequent alterations in ecosystem functions (Borken and Matzner, 2009; Jin et al., 2023; Zhang et al., 2020, 2023).

Carbon dioxide (CO_2) release from soil is an ecosystem process that is sensitive to DWCs (Birch, 1958; Borken and Matzner, 2009; Lee et al., 2002; Nagano et al., 2019; Unger et al., 2010, 2012; Zhang et al., 2020, 2023) and has substantial feedback potential to the ongoing climate change due to its magnitude reaching as much as seven times greater than anthropogenic CO_2 emission on a global scale (Bond-Lamberty and Thomson, 2010; Friedlingstein et al., 2020). The effects of DWCs on soil CO_2 release were first shown by Birch (1958) as the marked increase in soil organic matter (SOM) decomposition and CO_2 release after the rapid rewetting of dried soil, and has since been the subject of intensive investigation (Borken and Matzner, 2009; Kpemoua et al., 2023; Lee et al., 2002, 2004; Miller et al., 2005; Nagano et al., 2019; Unger et al., 2010, 2012; Xiang et al., 2008), including meta-analyses (Kim et al., 2012; Jin et al., 2023; Zhang et al., 2020). However, it is still difficult to precisely quantify and predict the effects of DWCs on soil CO_2 release.

The significant uncertainties in the effects of DWCs on soil CO_2 release include the inconsistent trends and sizes of effects likely due to the paucity of studies using constant moisture conditions equivalent to the mean water content during DWC incubation (Kpemoua et al., 2023; Zhang et al., 2020, 2023). According to a meta-analysis by Zhang et al. (2020) using 208 data from 34 sites in 29 reports, the effects of DWCs vary according to soil and water contents in continuous constant moisture conditions. Especially, changes in CO_2 release rate associated with DWCs ranged from -4% to +19% with an average of +4% in comparison with the medium level of constant moisture content~~equivalent to the~~, which should be the same with the mean water content during DWC incubation, whereas only 9 of 38 data representing CO_2 release rates ~~for conditions~~ were measured for such medium level of constant moisture ~~were measured~~ conditions (Zhang et al., 2020). Another 29 data were calculated ~~from the~~ as the average of two CO_2 release rates at the ~~upper and lower ends of the range of constant moisture content, corresponding to the~~ wettest and driest water contents of constant moisture conditions, which should be the same with the maximum and minimum water contents ~~of~~ in DWC treatment, respectively (Zhang et al., 2020). In the experiment using three Alfisols from Chinese long-term experimental field studies, Zhang et al. (2023) showed similar or somewhat lower CO_2 release in the DWC compared with the constant moisture conditions with the same mean water content for the DWC incubation. Using

two Luvisols from French long-term field experiment sites, Kpemoua et al. (2023) also showed similar features of changes in CO_2 release associated with DWCs, indicating the need for further comparison of CO_2 release between DWCs and constant moisture conditions with the same mean water content. In contrast to these studies, Nagano et al. (2019) found a 49% increase

55 in CO_2 release rate associated with DWCs in an Andisol collected from a Japanese forest~~, and this~~. This increase was more than double that of another non-volcanic ash soil from the same forest. ~~Therefore~~Thus, there are substantial variations in trends of effects of DWCs in comparison with constant moisture conditions ~~with~~ having the same mean water content during incubation, ~~and knowledge gaps remain~~ remaining knowledge gaps about environmental and soil predictors for variations in effect sizes. There are proposed roughly three mechanisms for CO_2 release increase by DWCs (Schimel, 2018; Barnard et al., 2020): (i)

60 increase in available carbon source via the releases of cellular metabolites from microbial cells destroyed by rewetting after the strong drought, (ii) increase in available carbon source by the releases of carbon from macroaggregates destroyed by repeated DWC, and (iii) changes in the microbial communities in response to transient moisture conditions. Nevertheless, there are still substantial knowledge gaps for critical mechanisms or the relative importance of those mechanisms among multiple soils.

~~The present study was performed~~ We perform the present study to evaluate overall trends in the effect of DWCs on soil
65 CO_2 release and to explore the predictors of variations in its effect size ~~in~~ among 10 Japanese forests and pastureland soils. These soils ~~were~~ are variously affected by volcanic ash during their pedogenesis ~~, andtherefore~~ and, therefore, include several Andisols, which are known to have a high SOM storage capacity (Morisada et al., 2004), likely due to the protection of SOM from microbial decomposition by ~~enrichment of~~ abundant reactive minerals and metals in these soils (Asano and Wagai, 2014; Imaya et al., 2007; Shirato et al., 2004). Reactive minerals and metals that contribute to the protection of SOM are iron
70 (Fe) and aluminum (Al), constituting short-range-order minerals and ~~organometal~~ organo-metal complexes (Asano and Wagai, 2014; Rasmussen et al., 2018; Shirato et al., 2004; Wagai et al., 2018). Although global coverage of Andisols is about 1% (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009), determination of the responses of carbon cycling in Andisols to DWCs will help in understanding the responses of non-volcanic ash soils, ~~where~~ because reactive minerals and metals are also essential in high ~~soil carbon stocks~~ carbon stocks of those soils (Rasmussen et al., 2018; Hall and Thompson, 2022) and may be sensitive to
75 climate and land use management, including water regime (Georgiou et al., 2022; Kramer and Chadwick, 2018).

2 Materials and methods

2.1 Site description and soil sampling

We collected 10 soil samples from depths of 0–5 cm or 0–10 cm in six forests and a pastureland located in Niigata (six soils from four forests), Ibaraki (two soils from a forest) (Nagano et al., 2019), and Oita (two soils from a forest and a pastureland)
80 (Wijesinghe et al., 2021) prefectures in Japan. Figure 1 and Table 1 present the locations and site characteristics, i.e., elevation, mean annual temperature (MAT), mean annual precipitation (MAP), potential evapotranspiration (PET), and net primary production (NPP). Briefly, all of the investigated sites have a humid temperate climate with MAT of 9.1–10.8°C and MAP of 1474–2930 mm. All of the forests are dominated by beech (*Fagus crenata* and *Fagus japonica*) and oak (*Quercus serrata*), except for Oita forest which is a deciduous/evergreen mixed forest dominated by Siebolds maple (*Acer sieboldianum*), Japanese
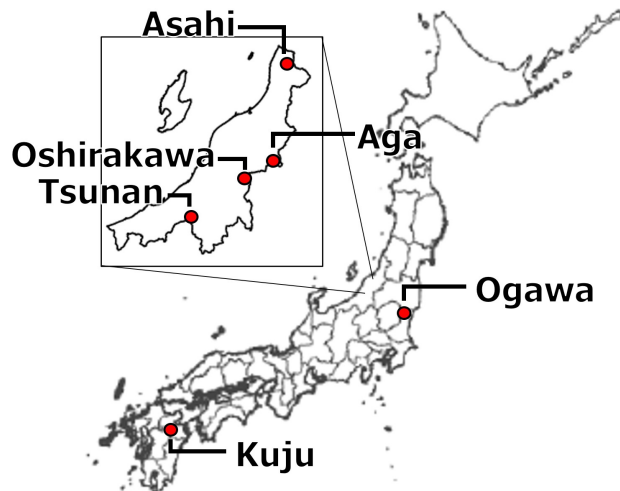
Figure 1. Locations of Japanese forests and pastureland where investigated soils were collected.

Table 1. Environmental properties of Japanese forests and pastureland where investigated soils were collected[a]

Prefecture	Soil	Latitude °N	Longitude °E	Elevation m	MAT °C	MAP mm	Annual PET mm	MAP-PET mm	Annual NPP g C m^{-2}
Niigata	Aga1, Aga2	37.55	139.51	474	10.6	1714	747	967	633
	Tsunan1, Tsunan2	37.04	138.60	716	10.8	1899	795	1104	628
	Asahi1	38.38	139.70	655	9.2	1887	697	1190	605
	Oshirakawa1	37.35	139.16	627	9.1	1963	668	1295	563
Ibaraki	Ogawa13, Ogawa 14	36.93	140.59	643	10.7	1474	764	710	785
Oita	Kuju_pasture, Kuju_forest	33.06	131.23	841	10.8	2930	786	2144	819

[a] MAT, MAP, and PET were obtained as averages for 1981–2020 in a global data set of climate and climatic water balance (i.e., TerraClimate) by Abatzoglou et al. (2018). NPP data were obtained as averages for 2001–2020 in the global distribution of NPP estimated from MODIS observation products (i.e., MOD17A3HGF) by Running and Zhao. (2021). MAT, mean annual temperature; MAP, mean annual precipitation; PET, potential evapotranspiration; NPP, net primary production.

snowbell (*Styrax japonicus*), and Japanese holly (*Ilex crenata*). The pastureland in Oita is dominated by Japanese lawn grass (*Zoysia japonica*), dwarf fountain grass (*Pennisetum alopecuroides*), cranesbill (*Geranium thunbergii*), white clover (*Trifolium repens*), and Indian strawberry (*Potentilla indica*).

Soil sampling was conducted in the snow-free season (April to October) of 2021. We also collected soil samples from layers below the target depth (i.e., 0–5 cm or 0–10 cm depth) down to 50 cm as the maximum depth to examine whether the soil could be classified as Andisol. According to the USDA (United States Department of Agriculture) Soil Taxonomy criteria (USDA Soil Survey Staff, 2022), soils with 60% or more of the thickness containing more than 20 mg of acid oxalate-extractable Al (Alo) plus 1/2 Fe (Feo) per 1 g soil within a depth of 0–60 cm are classified as Andisols. According to these criteria, 4 of the

10 soils were Andisols (one each from Niigata and Ibaraki, and two from Oita) with non-allophanic properties determined by a high ratio of pyrophosphate-extractable Al (Alp) content to Alo content (Alp/Alo > 0.5; USDA Soil Survey Staff (2022); Fig. S1). Collected soil samples were transferred to the laboratory and stored at 4°C before further analysis. Before analysis, soils were gently passed through a 4-mm sieve to remove gravel and plant tissue. Fine roots in the sieved samples were removed with tweezers. In our study, we considered that the soil water content at the soil sampling reflected the ability of soil to hold the water and thus the usual water contents in the field because the soil water content showed significantly positive correlations with water holding capacity (WHC) ($r = 0.87$, $p < 0.01$). Therefore, CO_2 release rate for constant moisture conditions in the present study should represent the release rate under the usual field moisture conditions of each soil.

2.2 Soil analysis

The soil properties analyzed were pH(H_2O), electrical conductivity, water content, water-holding capacity (WHC), total carbon (C) and nitrogen (N) contents, ~~and~~ particle size distributions as relative compositions of clay, silt, and sand-sized particles, and selectively dissolved Al and Fe contents (Tables 2 ~~and 3~~ – 4). The pH(H_2O) was measured in soil and water mixtures consisting of 1 g of soil and 2.5 mL of water. For measurement of electrical conductivity, 5 mL of water was added to 1 g of soil. Water content was measured by determining the difference in soil weight before and after drying at 105°C for 24 h. WHC was measured as the difference in soil weight before and after water saturation ~~.~~ referring to the Hilgard method (Mabuhay et al., 2003; Ahn et al., 2008). Here, water contents when soil is completely saturated in the Hilgard method should equal zero pF value (0 kPa) as soil water potential. Soil total C and N contents were measured for air-dried and well-ground soil samples using an elemental analyzer (vario PYRO cube; Elementar, Manchester, UK). Particle size distributions were determined using the Stokes' law-based sedimentation method (Miller et al., 1988; Nakai, 1997) using soil mineral particles after removing organic matter by hydrogen peroxide solution digestion. Selectively dissolved metals such as reactive Al and Fe were measured according to the procedure described previously ~~Nagano et al. (2023). The~~ in Nagano et al. (2023). Briefly, the contents of Al and Fe extractable with 2.0 M acid ammonium oxalate (i.e., Alo and Feo, respectively) were measured as contents of ~~organometal~~ organo-metal complexes and short-range-order minerals, while Al and Fe extractable with 0.1 M sodium pyrophosphate solution (i.e., Alp and Fep, respectively) were measured as contents of ~~organometal~~ organo-metal complexes (Asano and Wagai, 2014; Takahashi and Dahlgren, 2016; Wagai et al., 2018). The difference between acid oxalate- and pyrophosphate-extractable metals (i.e., Alo-p and Feo-p) represented the contents of short-range-order minerals (Courchesne and Turmel, 2008). Contents of Al and Fe in the solution were measured with an inductively coupled plasmaoptical emission spectrometer (ICP-OES) (5110; Agilent Technologies, Santa Clara, CA, USA). Carbon concentrations in pyrophosphate-extracted solution (Cp) were also measured with a total organic carbon (TOC) analyzer (TOC-L; Shimadzu, Kyoto, Japan). For soils from Kuju forest and grassland and two Ogawa forests, we also measured C concentration in free light density fraction (fLF) (Leuthold et al., 2023) obtained by the density fractionation method using sodium polytungstate solution having 2.0 g cm^{-3} (Koarashi et al., 2012), in order to evaluate possible effects of labile C abundance in fLF on CO_2 release increase by DWCs.

Table 2. ~~Soil~~ Basic properties of 10 Japanese forests and pastureland soils collected from depths of 0–5 or 0–10 cm

Soil	Soil type[a]	pH(H$_2$O) ~~Total N~~	EC ~~Total C/N~~	Water content	WHC	~~Total C~~	Particle size-distribution	
						Sand	Silt	Clay
			μS cm^{-1}	g water g^{-1} soil	g water g^{-1} soil		% ~~%~~	
Aga1	Inceptisols or Entisols	4.36	23	0.77	0.80	~~8.8~~ 27	~~0.56~~ 30	~~15.7~~ 43
Aga2	Inceptisols or Entisols	4.39	27	1.07	1.12	~~14.3~~ 17	~~0.79~~ 59	~~18.2~~ 23
Tsunan1	Inceptisols (Brown forest soils)	4.72	17	0.78	1.06	~~8.8~~ 47	~~0.63~~ 44	~~19.8~~ 9
Tsunan2	Inceptisols (Brown forest soils)	4.08	39	1.06	1.32	~~14.3~~ 34	~~0.98~~ 48	~~15.6~~ 18
Asahi1	Inceptisols (Brown forest soils)	4.07	39	1.70	2.14	~~23.7~~ 29	~~1.41~~ 41	~~16.9~~ 30
Oshirakawa1	Andisols	4.66	24	1.07	1.26	~~15.1~~ 38	~~0.76~~ 45	~~19.8~~ 17
Ogawa13	Inceptisols (Brown forest soils)	5.29	18	0.46	1.00	~~6.8~~ 66	~~0.31~~ 25	~~22.2~~ 8
Ogawa14	Andisols	5.30	27	1.14	1.68	~~16.1~~ 68	~~0.86~~ 23	~~18.6~~ 9
Kuju_pasture	Andisols	5.35	133	1.32	1.43	~~21.8~~ 72	~~1.37~~ 22	~~15.9~~ 5
Kuju_forest	Andisols	3.80	168	1.49	1.58	70	25	4

[a] Andisols were determined according to the USDA Soil Taxonomy criteria (USDA Soil Survey Staff, 2022) based on acid oxalate-extractable Al plus 1/2 Fe contents (see text for details). All Andisols were non-allophanic. Other soil types were determined using a Japanese soil digital map, i.e., Japan Soil Inventory NARO (2023).

For the soils after incubation (see Section 2.3), soil microbial biomass C and N were measured by the chloroform fumigation-extraction method (Vance et al., 1987). Organic C and total N concentrations in 0.5 M potassium sulfate solution used for extraction of fumigated and nonfumigated soils were measured using another TOC analyzer (TOC-L; Shimadzu) equipped with a total nitrogen (TN) unit (TNM-L, Shimadzu).

2.3 Incubation experiment and soil CO$_2$ release rate measurement

Soils were incubated aerobically at 20°C for 84 days including three DWCs (i.e., 28 days per cycle). Simultaneously, soils were incubated in the same manner but without DWCs, during which water content of the soils was maintained at a constant level equivalent to the mean water content for the DWC treatment (Fig. 2). A pre-incubation was conducted at the constant water content for 7 days prior to the 84-day incubation. A post-incubation was also conducted at the constant water content for 28 days after the 84-day incubation to evaluate the remaining effect of DWCs on soil CO$_2$ release. We consider that the

Table 3. Soil carbon (C) and nitrogen (N) properties

Soil	Total C	Total N	Total C/N	C in free light density fraction (fLF)
~	%	%		% to total C
Aga1	8.8	0.56	15.7	No data
Aga2	14.3	0.79	18.2	No data
Tsunan1	12.4	0.63	19.8	No data
Tsunan2	15.3	0.98	15.6	No data
Asahi1	23.7	1.41	16.9	No data
Oshirakawa1	15.1	0.76	19.8	No data
Ogawa13	6.8	0.31	22.2	10.0
Ogawa14	16.1	0.86	18.6	10.0
Kuju_pasture	21.8	1.37	15.9	14.0
Kuju_forest	22.3	1.18	18.9	5.9

Table 4. Selectively dissolved minerals and associated carbon contents in soils

Soil	Acid oxalate-extractable metals			Pyrophosphate-extractable metals			Acid oxalate – Pyrophosphate			Alp/Alo	Pyrophosphate-extractable C
	Al	Fe	Al+0.5Fe	Al	Fe	Al+0.5Fe	Al	Fe	Al+0.5Fe		
	mg g^{-1} dry soil			mg g^{-1} dry soil			mg g^{-1} dry soil				mg g^{-1} dry soil
Aga1	3.3	7.9	7.2	2.8	5.0	5.3	0.5	2.8	2.0	0.84	21.0
Aga2	6.9	10.6	12.2	6.8	10.5	12.0	0.1	0.2	0.2	0.98	42.9
Tsunan1	8.9	13.8	15.8	8.5	11.3	14.2	0.4	2.5	1.6	0.96	39.9
Tsunan2	6.9	8.5	11.1	7.1	7.2	10.6	-0.2	1.4	0.5	1.03	47.4
Asahi1	4.8	10.2	9.9	4.7	9.2	9.3	0.1	1.0	0.6	0.98	45.4
Oshirakawa1	9.5	12.4	15.7	9.1	11.0	14.6	0.4	1.4	1.2	0.95	43.0
Ogawa13	9.2	8.7	13.5	6.9	6.5	10.2	2.3	2.2	3.4	0.75	19.4
Ogawa14	24.9	16.6	33.2	23.2	12.7	29.6	1.6	3.9	3.6	0.93	59.1
Kuju_pasture	22.0	20.3	32.2	19.0	14.1	26.0	3.1	6.3	6.2	0.86	96.7
Kuju_forest	19.5	19.1	29.0	19.0	16.2	27.1	0.4	2.9	2.0	0.98	109.8

incubated soils have been aerobic even after the rewetting to increase the water content by twice the WHC, because the CO_2 concentrations in our experiment never overwhelmed 1%, thus the oxygen concentrations in the incubation jar have likely never
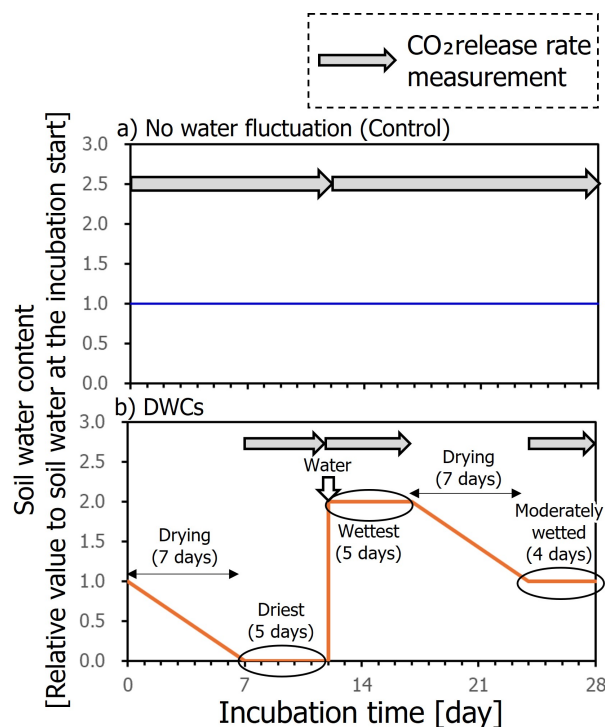
Figure 2. Schematic time courses of soil moisture during incubation with constant moisture content (~~upper panel~~a) and DWCs (~~bottom panel~~b) with measurement period of CO_2 release rate.

decreased below 19% or lower. Also, a sufficiently large volume of our incubation jar (1.0L) against contained soil amounts (i.e., 5.31-10.63 g) and added water contents in the rewetting (i.e., ca. 6 to 7 mL) support the state of aerobic condition during

140 the incubation.

Mason jars (1.0 L volume; Ball, Buffalo, NY, USA) with lids equipped with tube fitting systems for gas sample collection (Koarashi et al., 2012; Nagano et al., 2019) were used as incubation jars. Small vials (300 mL, SM sample glass vial; Sansyo, Tokyo, Japan) containing 5.31–10.63 g of soil sample depending on water content were placed in Mason jars. Under the constant water treatment, we surrounded the small vial with 20 mL of water within the incubation jar to prevent the soil from

145 drying. For each DWC, Day 1 to Day ~~8~~ 7 and Day 18 to Day ~~25~~ 24 were drying stages (Fig. 2), during which the soils were incubated with silica gel (20 g jar^{-1}), which lowered the water content to < 5% WHC by Day ~~8.~~ 7. Day 8 to Day ~~13~~12, Day 13 to Day ~~18~~17, and Day 25 to Day ~~29~~ 28 were the driest, wettest, and moderately wet stages, respectively, and CO_2 release rates in these three stages were measured using a gas chromatograph equipped with a thermal conductivity detector (GC-14B; Shimadzu). The CO_2 release rates were also measured in the pre- and post-incubation periods. At the beginning of the wettest

150 stage, soils were rapidly rewetted with distilled water to double the soil water content from the initial status of DWC incubation. For CO_2 release rate measurements, at the start of each stage, the headspace of the incubation jar was flushed with CO_2-free

air for 15 min at a rate of 0.5 L min^{-1} and the jar was closed. At the end of each stage, 15 mL of gas sample was collected from the jar using a 20-mL plastic syringe (Terumo, Kyoto, Japan) and stored in a pre-evacuated 5-mL glass vial (SVG-5; Nichiden Rika, Osaka, Japan). Then, the CO_2 release rate was determined from the increase in CO_2 concentration during this period.

155 After gas sampling, the jars were flushed with CO_2-free air and closed for the next incubation stage. ~~Incubations~~ For 84 days in the incubations with the constant ~~water content were conducted~~ moisture condition as controls, ~~where~~ the CO_2 release rates were measured for Day 1 to Day 12, Day 13 to Day 28, Day 29 to Day 40, Day 41 to Day 56, Day 57 to Day 68, and Day 69 to Day 84, in addition to the pre- and ~~Day 13 to Day 29.~~ post-incubation periods. Soil water contents during the incubation were measured periodically and maintained by adding water to ensure the same mean water content between the two treatments.

160 Even in the drying stage under the DWC treatment for Day 1 to Day 7 and Day 18 to Day 24, we conducted measurements of soil water content once to twice. The measurements were performed by weighing those soils. Based on these data, we confirmed that the mean soil water content during DWC incubation was equal to that during constant moisture incubation. All incubations were conducted with three replicates for each treatment and soil.

2.4 Data processing and statistical analysis

165 The CO_2 release rates were compared between the DWC and constant water content treatments. For the DWC treatment, the CO_2 release rates in the drying stages (i.e., Day 1 to Day ~~8~~ 7 and Day 18 to Day ~~25~~ 24) could not be measured and therefore had to be estimated to evaluate the mean CO_2 release rates for the individual cycle and total of three cycles. The rates in the drying stages were estimated as the mean values of the CO_2 release rates measured before and after the period of interest, in the same manner as described previously (Nagano et al., 2019). Then, the effect size of the DWCs on CO_2 release (defined

170 here as the increase factor, IF_{CO2}) was quantified as the ratio of CO_2 release rate under the DWC condition to that under the constant water content condition (Nagano et al., 2019; Zhang et al., 2020). Here, doubling of CO_2 release by DWC resulted in IF_{CO2} of 2, while halving resulted in IF_{CO2} of 0.5.

The pairwise *t* test was applied to examine the statistical significance of differences in CO_2 release rates between the DWC and constant water content treatments with adjustment of site-by-site variations in the metrics. Differences in soil microbial

175 biomass C and N between the two treatments were also evaluated with the pairwise *t* test. To explore predictors explaining the variation in IF_{CO2} among soils, relations between IF_{CO2} and environmental and soil properties were visualized on scatter plots and evaluated by linear correlation analysis. All statistical analyses were conducted with R 4.1.1 ~~(?),~~ (R Core Team, Vienna, Austria), and $p < 0.05$ was taken to indicate statistical significance.

3 Results

180 ### 3.1 Quantifying the effect of DWCs on soil CO_2 release

The CO_2 release rates under DWC conditions showed large fluctuations for all soils along with fluctuations in soil water content (Fig. 3). The CO_2 release rates in the driest stages (3.5%–18.2% of WHC depending on the soil) were 3.0–41.5 μg C g^{-1} dry
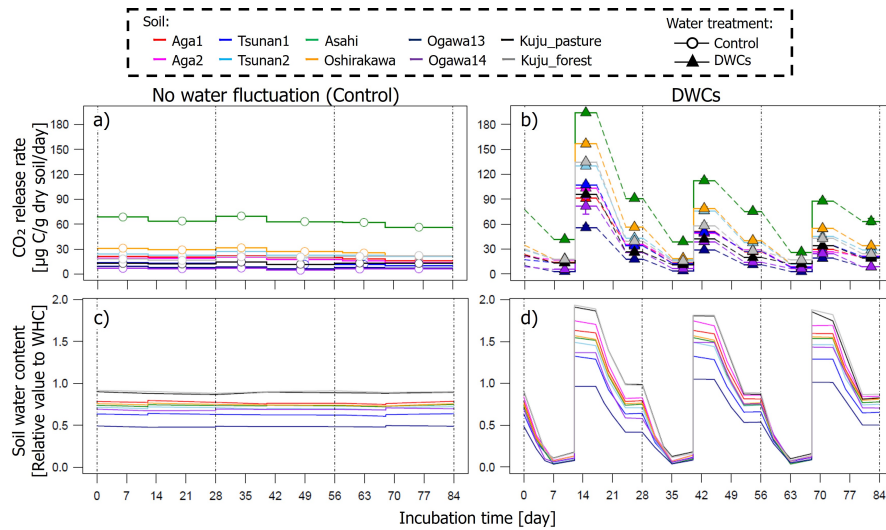
Figure 3. Time courses of soil CO_2 release rates (~~upper panels~~a and b) and water contents (~~bottom panels~~c and d) during 84-day incubation under constant water content (a and c) and three DWC conditions (b and d).

soil day^{-1}. In contrast, CO_2 release rates in the wettest stages (90.8%–201.1% of WHC) reached 18.8–194.1 μg C g^{-1} dry soil day^{-1}. In the moderately wet stages, CO_2 release rates were 8.4–63.9 μg C g^{-1} dry soil day^{-1}. For the constant water content
185 treatment, CO_2 release rates observed for each soil showed little fluctuation during incubation although the rates varied from 7.3 to 69.9 μg C g^{-1} dry soil day^{-1} depending on the soil.

For all soils, the observed large fluctuations in CO_2 release under DWC treatment resulted in a greater CO_2 release rate than under constant water content treatment, although the IF_{CO2} values varied among the soils (Fig. 4). At the wettest stage in the first DWC, the CO_2 release rates increased by 47.2–127.7 μg C g^{-1} dry soil day^{-1} compared with those under the constant
190 water content conditions, resulting in IF_{CO2} values of 2.9–12.2. For the whole of the first cycle (i.e., the first 28 days), the CO_2 release rates increased by 15.4–43.4 μg C g^{-1} dry soil day^{-1} under the DWCs compared with the constant water content conditions, resulting in IF_{CO2} values of 1.6–5.2. For the whole incubation period (84 days) including three DWCs, IF_{CO2} values were 1.3–3.7, with an increase in CO_2 release rate by 7.4–23.8 μg C g^{-1} dry soil day^{-1} by DWCs. These increases in CO_2 release by DWCs were observed in all cycles during the 84-day incubation period, whereas no increase was observed
195 in the 28-day post-incubation period after the three DWCs (Fig. S2). ~~Taking these results into consideration~~Considering these results, we focused on the IF_{CO2} ~~values~~variations obtained for the whole incubation period, including three DWCs.

3.2   Exploring predictors of the effect sizes on the increase in CO_2 release

Among the environmental and soil physiochemical properties, reactive mineral and metal contents (~~i.e.~~especially, Alo+0.5Feo, Alo, Feo, Alp+0.5Fep, and Alp) in ~~soil, other than Fep, Alo-p+0.5Feo-p, Alo-p, and Feo-p,~~ soils showed significant positive
200 correlations with IF_{CO2} ($p < 0.05$; Table ~~4~~5). In particular, Alp content appeared to be a key predictor of the variation in IF_{CO2}
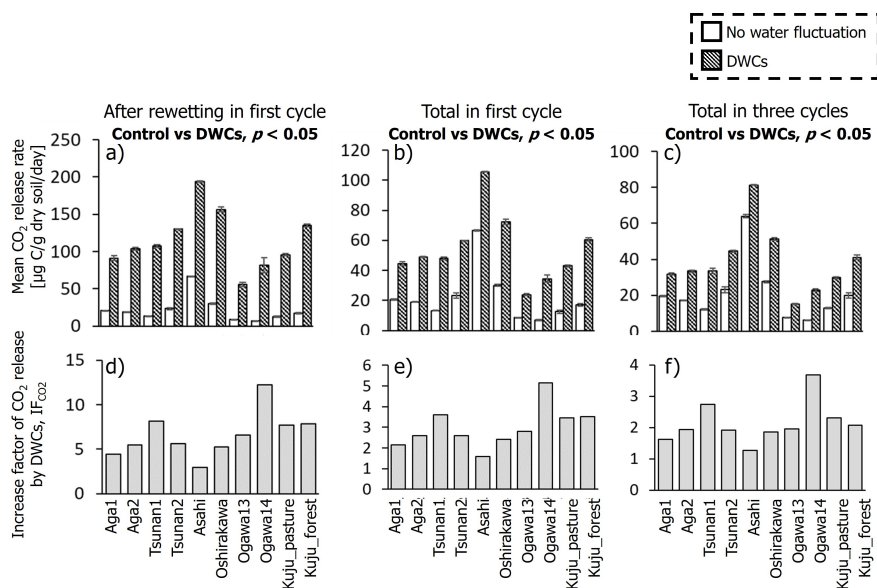
Figure 4. Comparisons of mean CO_2 release rates after rewetting in the first cycle, the whole of the first cycle, and for the total of three cycles between DWC and constant water content conditions (~~upper panels~~a-c), and the factor of increase in CO_2 release by DWCs (IF_{CO2}) for individual periods (~~bottom panels~~d-f). Statistically significant differences ($p < 0.05$, pairwise t test) in CO_2 release rate between the two treatments are presented.

among the soils, given that Alp accounted for most (73%–99%) of Alo (Table ~~3~~4) and showed a higher correlation coefficient than ~~Feo (Table 4~~others (Table 5). Scatterplots for Alp content and IF_{CO2} values are presented in Fig. 5. In addition, the molar ratios of soil total C and Cp contents to Alp contents showed significant negative correlations to IF_{CO2} ($p < 0.05$; Table ~~4~~5, Fig. 5). Here, it should be noted that IF_{CO2} had no significant relationship with soil water content at soil sampling and WHC, suggesting the variation in IF_{CO2} among soils resulting from other than such hydrogenic properties of soils. The SOM quality, such as C/N ratios of total bulk soils and K_2SO_4 extractable fractions, also had no signifcant relationship with IF_{CO2}. The insignificant relations of SOM quality to IF_{CO2} were also supported by the facts that Kuju forest and grassland soils having more than doubling differences in fLF abundance showed only 12% differences in IF_{CO2}, and two Ogawa forest soils having almost identical fLF abundances showed more than doubling differences in IF_{CO2}. The amounts of clay and sand-sized particles showed significant correlations with IF_{CO2} after rewetting in the first cycle ($p < 0.05$, $r = -0.66$ for clay and 0.71 for sand particles). However, those correlations between the particle contents and IF_{CO2} were insignificant for IF_{CO2} for a total of three cycles. Thus, in the present study, reactive mineral and metal content, especially Alp content in soils, was the significant predictor for IF_{CO2} variation among soils, rather than soil water environments, SOM quality, and particle size distributions.

Among the additional soil properties related to soil microbial activity and abundance, the soil C content-specific CO_2 release rate under constant moisture conditions (defined as the CO_2 release rate per unit of C in soil, qCO2_soc, in $\mu g\ CO^2\text{-}C\ mg^{-1}$ soil-C day^{-1}) showed significant negative correlations with IF_{CO2} values in all incubation stages ($p < 0.01$; Table ~~5~~6, Fig. 6).

Table 5. Pearsons correlation coefficients between IF_{CO2} and environmental and soil properties

Environmental or soil property	After rewetting in first cycle	Total in first cycle	Total in three cycles
Elevation	0.36	0.37	0.22
MAT	0.54	0.55	0.46
MAP	0.05	0.08	-0.11
MAP-PET	0.01	0.04	-0.14
Water content at sampling	-0.11	-0.07	-0.15
WHC	0.06	0.08	0.04
pH(H_2O)	0.54	0.51	0.57
Electronic conductivity	0.19	0.21	-0.01
Total C	-0.03	0.01	-0.11
Total N	-0.07	-0.03	-0.12
C/N ratio	0.27	0.23	0.22
Sand	**0.72***	**0.70***	0.56
Silt	-0.50	-0.48	-0.35
Clay	**-0.69***	**-0.68***	-0.57
Alo+0.5Feo	**0.81****	**0.82****	**0.70***
Alo	**0.84****	**0.85****	**0.73***
Feo	**0.64***	**0.66***	0.53
Alp+0.5Fep	**0.81****	**0.82****	**0.70***
Alp	**0.84****	**0.85****	**0.74***
Fep	0.55	0.58	0.54
Alo-p+0.5Feo-p	0.46	0.43	0.37
Alo-p	0.56	0.57	0.48
Feo-p	0.55	0.55	0.46
Total C/Alp molar ratio	**-0.79****	**-0.78****	**-0.72***
Cp	0.39	0.42	0.23
Cp/Alp molar ratio	**-0.79****	**-0.75***	**-0.74***

Here qCO$_2$_soc should be an index for microbial availability of carbon substrate normalized by total C contents in soils. Thus, using qCO$_2$_soc, we can consider whether the microbially available carbon substrate in interested soil should be much more
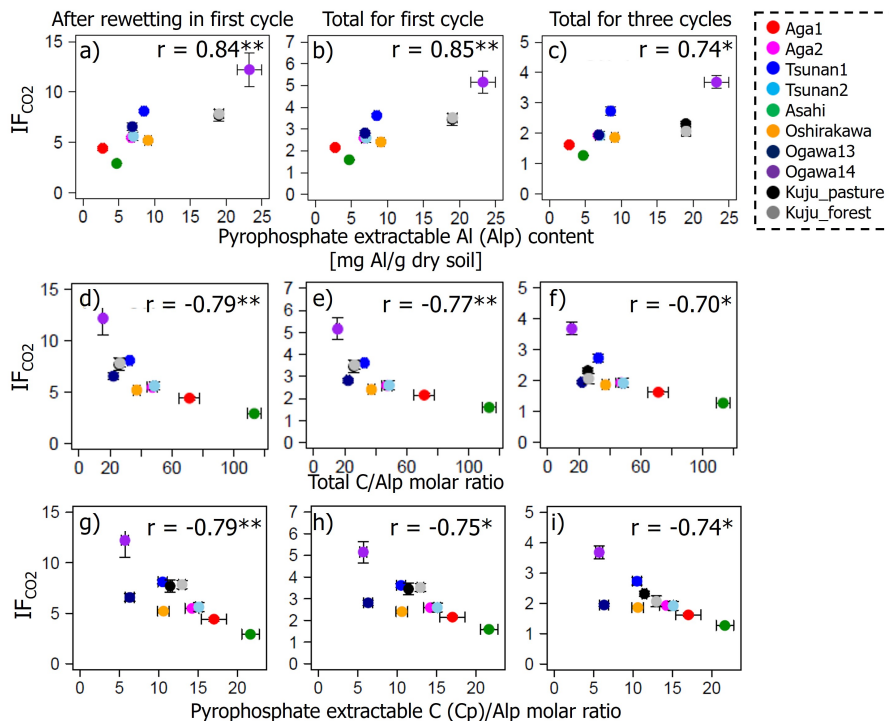
Figure 5. Relations between IF_{CO2} and soil pyrophosphate-extractable Al (Alp) content (upper panels), total C to Alp molar ratio (middle panels), and pyrophosphate-extractable C (Cp) to Alp molar ratio (bottom panels). Significant correlation coefficients at $p < 0.01$ and $p < 0.05$ are indicated with single (*) and double asterisks (**), respectively.
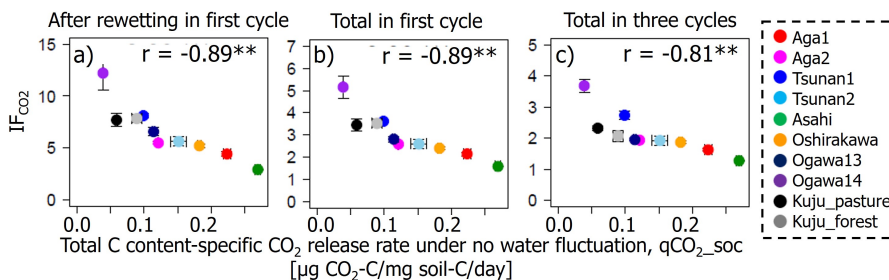
Figure 6. Relations between IF_{CO2} and total C content-specific CO_2 release rate under constant moisture content (qCO2_soc). Significant correlation coefficients at $p < 0.01$ and $p < 0.05$ are indicated with single (*) and double asterisks (**), respectively.

than in other soils. Microbial biomass N also showed ~~significant~~ significantly negative correlations with IF_{CO2} values after rewetting in the first cycle and in the whole of the first cycle ($p < 0.05$) but not in the whole incubation period including three cycles ($p = 0.12$).

Table 6. Pearsons correlation coefficients between IF_{CO2} and soil microbial properties

Soil microbial property	After rewetting in first cycle	Total in first cycle	Total in three cycles
Total C content-specific CO_2 release rate under no water fluctuation, qCO_2_soc	**-0.87***	**-0.86****	**-0.77***
K_2SO_4 extractable C	-0.25	-0.21	-0.33
K_2SO_4 extractable N	-0.63	-0.60	-0.55
K_2SO_4 extractable C/N	0.15	0.18	-0.01
Microbial biomass C	-0.62	-0.61	-0.48
Microbial biomass N	**-0.67***	**-0.66***	-0.52
Microbial biomass C/N	0.47	0.48	-0.31
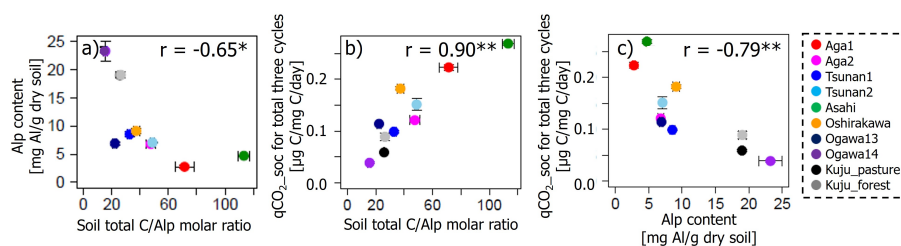
Figure 7. Relations among soil Alp contents, total C/Alp molar ratio, and qCO_2_soc. Significant correlation coefficients at $p < 0.01$ and $p < 0.05$ are indicated with single (*) and double asterisks (**), respectively.

There were also considerable relations among Alp contents, total C/Alp molar ratio, and qCO_2_soc (Fig. 7). Soil Alp content and total C/Alp molar ratio showed a ~~trend toward a positive correlation , although the~~ negative correlation at p ~~value was 0.06~~< 0.05. The qCO_2_soc showed a positive correlation with total C/Alp molar ratio and negative correlation with Alp content at p

225 < 0.01. The Cp/Alp molar ratio also showed similar covariations with these variables, showing a strongly positive correlation with total C/Alp molar ratio ($r = 0.90$, $p < 0.01$; Fig. S3).

It should be noted that both microbial biomass C and N were significantly lower in soils incubated under DWC conditions than constant water content conditions ($p < 0.05$; Fig. 8). Microbial biomass C under DWC conditions was lower by 20.2% than those under constant water content conditions. Microbial biomass N under DWC conditions was 12.6% lower than under

230 constant water content conditions. Nevertheless, there were no significant correlations between the differences in microbial biomass and the value of IF_{CO2} (p 0.15; Fig. 9).
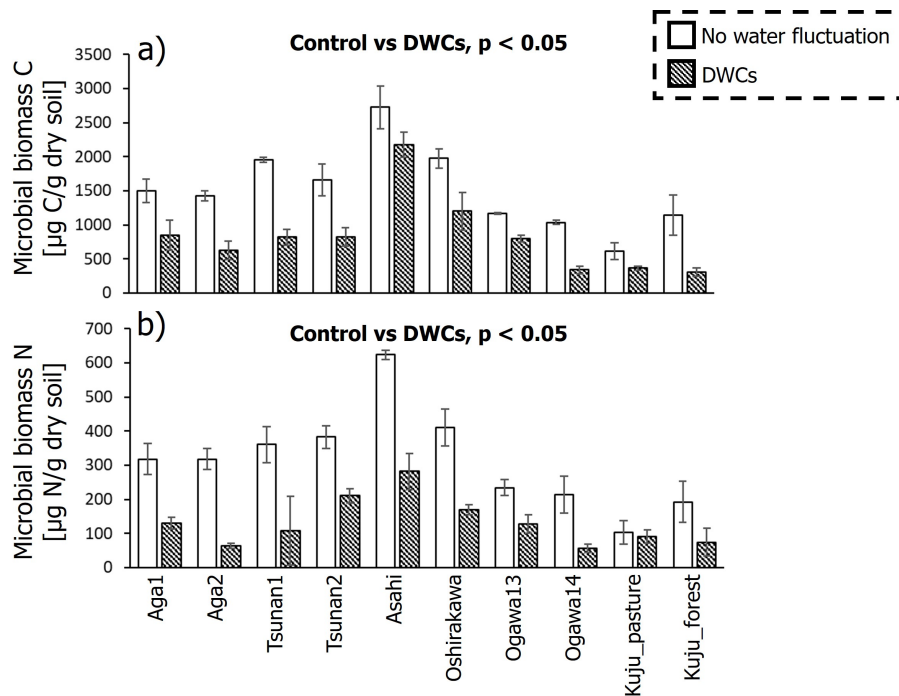
Figure 8. Comparisons of microbial biomass C (upper panel) and N (bottom panel) after incubation between DWC and constant water content conditions. Significant differences ($p < 0.05$ by pairwise t test) in microbial biomass between two treatments are presented.

4 Discussion

An increase in CO_2 release due to DWCs was consistently observed across 10 forest and pastureland soils in Japan (Figs. 3 and 4). The comprehensive increases in CO_2 release by DWCs were different from the findings of a recent meta-analysis of
235   studies showing no significant increases in CO_2 release under DWCs compared with constant water content with an equivalent mean water content during the period of interest (Zhang et al., 2020). Furthermore, our observations quantified the increase in CO_2 release due to three DWCs as IF_{CO2} values of 1.3–3.7. The observed effect size of DWCs on the CO_2 release from soils was large given that even a 20% increase in CO_2 release from the worlds soils can exceed the annual CO_2 emission from anthropogenic processes (Friedlingstein et al., 2020). ~~Especially, the importance of Alp for variations in~~
240   Furthermore, analyzing the relations between IF_{CO2} ~~(Table 4, Fig. 5; discussed below) indicated the vulnerability of Andisols covering about 1% of global land area (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009). In addition to Andisols, Spodic horizons in Podzols are often abundant in Alp (Ferro-Vázquez et al., 2020; Mokma, 1983). Therefore, Alp-rich soils cover approximately 5% of the global land area (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009). As these Alp-rich soils sometimes store much greater amounts of C than other soils (Asano and Wagai, 2014; Imaya et al., 2007; Morisada et al., 2004; Shirato et al., 2004)~~
245   ~~, the vulnerability of SOM protection by Alp offers significant insight for quantifying feedback of terrestrial C cycling to climate change.~~
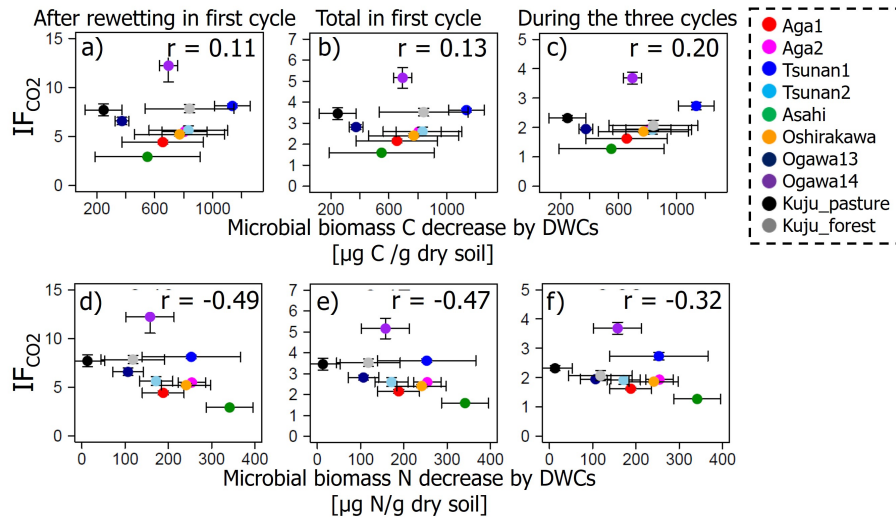
Figure 9. Relations between IF_{CO2} and microbial biomass C or N decrease by DWCs. There were no statistically significant correlations ($p > 0.05$).

~~A significant positive correlation was found~~ and fundamental soil properties showed a significantly positive correlation between IF_{CO2} and soil Alp content ~~by analyzing the relations between IF_{CO2} and fundamental soil properties (Table 4~~(Table 5, Fig. 5). ~~Here, soil Alp content was different between non-volcanic ash soils in previous meta-analyses (Zhang et al., 2020; Jin et al., 2023)~~

250 ~~and the present study in Japanese forest and pastureland soils. Of the organometal~~ Of the organo-metal complexes measured as pyrophosphate-extractable metals, Fep is known to be sensitive to DWCs, especially in seasonally flooded forests and wetlands, likely due to their vulnerability to redox potential changes caused by alterations to the water regime (Lacroix et al., 2019; Chen et al., 2017, 2018; Chen and Thompson, 2018). Less is known about the vulnerability of Alp to DWCs. However, soil Alp content may be affected by DWCs through changes in soil acidity. A previous field survey conducted in Japanese forest and arable

255 soils by Takahashi et al. (2006) showed that liming of non-allophanic Andisols increased soil pH and decreased Alp content. Miyazawa et al. (2013) verified this behavior by a laboratory incubation experiment for Andisols. Although we did not monitor pH during incubation in the present study, increases in pH after DWCs have been widely observed in upland agricultural (Wang et al., 2020; Meng et al., 2020), seasonally submerged paddy, and wetland soils (Majumdar et al., 2023). Therefore, the acidity mitigation by DWCs would destroy the organo-Al complexes, and increase microbially available C through the release of C

260 protected by the organo-Al complexes or other soil elements, such as macro- and microaggregates, which are tightly bonded by organo-Al complexes and thus physically protect organic C from microbial decomposition (Asano and Wagai, 2014; Takahashi and Dahlgren, 2016; Wagai et al., 2018).

Covariations among soil Alp contents, total C/Alp molar ratio, and qCO_2_soc (Fig. 7) also support the state of soil Alp content as the primary predictor of variations in IF_{CO2}. Negative correlations of soil Alp contents with both total C/Alp molar

265 ratio and qCO_2_soc suggested that more Alp to total C (i.e., high total C/Alp values) strengthens binding between Alp and

organic matter, resulting in resistance of organic matter to microbial decomposition under constant water conditions (i.e., low qCO_2_soc values). These features among reactive Al and SOM dynamics would result in positive correlations of qCO_2_soc and total C/Alp molar ratio with IF_{CO2} (Table 56, Figs. 5 and 6). However, such ability of Alp-rich soils to protect SOM from microbial decomposition likely does not persist under conditions of increased water fluctuations associated with DWCs, as suggested above. Given that amounts of pyrophosphate extractable C (Cp) in investigated soils (>19,000 tg/g dry soil, Table 4), which represented C associated with the organo-metal complex, were substantially greater than observed CO_2 release increase by DWCs (620–1999 μg C g^{-1} dry soil 84 days^{-1}), limited and specific C substrates in the organo-metal complex likely associated with CO_2 increase by DWCs.

We also found substantially lower microbial biomass in soils subjected to DWCs than constant water content conditions (Fig. 8), suggesting a decrease in microbial biomass through the destruction of microbial cells by DWCs (Kaiser et al., 2015; Marumoto et al., 1977, 1982; Marumoto, 1984; Nagano et al., 2023; Unger et al., 2010, 2012). The destruction of microbial cells is expected to release soluble organic matter available for microbes that have survived the DWC and to cause a marked increase in CO_2 release after rewetting (Marumoto et al., 1977, 1982; Marumoto, 1984; Nagano et al., 2019; Unger et al., 2010, 2012). Nevertheless, the contribution of microbially derived substances to the increase in CO_2 release remained unclear in the present study because of the lack of a significant correlation between IF_{CO2} and the decrease in microbial biomass (Fig. 9). There Whereas there was also no significant correlation between the decrease in microbial biomass and the increase in CO_2 release due to DWCs (Fig. S3), whereas S4), the decrease in microbial biomass C (246–1134 μg C g^{-1} dry soil) was within the amount of increase in CO_2 release (620–1999 μg C g^{-1} dry soil 84 days^{-1}). Furthermore, such changes in microbial biomass likely affect the microbial community structure and have the potential to alter the dynamics of other greenhouse gases (e.g., methane and nitrous oxide) , which are driven by specific microbes and are also known to be sensitive to DWCs (Kim et al., 2012; Jin et al., 2023)Therefore, the strict mechanisms of these carbon sources to CO_2 release increase, including the persistence and timing of their contribution, still require further works (Schimel, 2018; Barnard et al., 2020), considering the significant contribution of more than two carbon pools to the CO_2 release increase (Slessarev and Schimel, 2020; Warren and Manzoni, 2023), as well as carbon likely released by destructions of organo-metal complexes and microbial biomass by DWC as suggested in the present study.

It should be noted that shortcomings from unmeasured CO_2 release during the drying periods for DWCs treatments (i.e., Day 1 to Day 7 and Day 18 to Day 24 in each cycle) should be minor, while the linear changes in CO_2 release rate during the drying also assumed in other studies (e.g., Nagano et al. (2019); Zhang et al. (2020); Jin et al. (2023)). This is because we observed significant relationships between IF_{CO2} and organo-Al complex also in IF_{CO2} after the rewetting in addition to the IF_{CO2} for total 1st cycle and three cycles (Table 5).

5 Conclusions

The present studyprovides significant insights into the precise quantification of the effects of DWCs on soil CO_2 release on regional and global scales. A Through the present study, a comprehensive increase in CO_2 release by DWCs (i.e., 1.3–3.7-

fold greater than CO_2 release under constant water conditions) was observed in Japanese forest and pastureland soils. These magnitudes of increase in CO_2 release were strongly correlated with soil Alp content, total C/Alp molar ratio, and total C content-specific CO_2 release rate under constant water conditions (i.e., qCO$_2$_soc), suggesting the possible vulnerability of SOM protection by organo-Al complexes against DWCs. A decrease in microbial biomass by DWCs was also suggested, whereas their ~~relation~~ relations with the increase in CO_2 release remains to be determined in future studies.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions. YS and HN established the basic research design, conducted all data analyses, including software preparation, validation, and visualization, and wrote the original manuscript. SH, MA-A, JK, and HN conducted soil sampling. YS, SH, MA-A, JK, and HN contributed to the detailed research design, soil analysis, data validation, interpretation of the results, and editing of the manuscript. TY and YK provided essential support in analysis of soil properties and data. All authors contributed to editing the article and approved the submitted version.

Competing interests. The authors declare that they have no known competing financial interests or personal relations that could have appeared to influence the work reported in this paper.

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