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Stable C and N isotope abundances in water-extractable organic matter from air-dried soils as potential indices of microbially utilized organic matter

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Stable carbon (C) and nitrogen (N) isotopes (^{13}C and ^{15}N) in water-extractable organic matter (WEOM) derived from air-dried soils may be applicable to elucidate the microbial decomposition of soil organic matter (SOM), which is crucial in terrestrial C cycles. A total of 40 soil samples were collected from a depth of 0–6 cm from a temperate broadleaved forest in Japan with vegetation succession from grassland approximately 150 years ago. Those soil samples were air-dried before the water extraction process and organic matter analysis. The C and N concentrations of WEOM were <3.6% of those of the bulk soil and were positively correlated with those of the bulk soil at a p-value of < 0.01. A positive correlation between the two fractions (i.e., WEOM and bulk soils) was also found for natural ^{13}C and ^{15}N abundances (δ^{13}C and δ^{15}N; $p < 0.01$). However, the C/N ratio of WEOM was slightly correlated with that of bulk soils, exhibiting a narrow range of values of ~10. Thus, those features of the WEOM were similar to the well-known features of microbial biomass. The δ^{13}C and δ^{15}N enrichments in WEOM relative to bulk soil, the difference in stable isotope abundances between bulk SOM and WEOM were negatively and positively correlated, respectively, with the concentrations of organo-mineral complexes and short-range order minerals (non-crystalline oxyhydroxides of aluminum and iron, allophane, imogolite, and allophane-like constituents), which play significant roles in SOM stabilization in soils. These relationships suggest that the stable isotopic enrichments in WEOM can be a good indicator of the microbial utilization of soil C and N under different substrate availabilities, which are crucial to SOM decomposition and decomposability substantially varying from local to global scales.

KEYWORDS

microbial decomposition, soil organic matter, stable isotopic signatures, substrate availability, water-extractable organic matter

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1. Introduction

An analysis of soil microbial biomass may shed light on which carbon (C) substrates are practically used in soils (Wieder et al., 2013; Wei et al., 2016), which are crucial components driving the major carbon dioxide (CO_2) efflux in terrestrial ecosystems (Davidson and Janssens, 2006; Bond-Lamberty et al., 2016, 2018; Luo et al., 2016). This is because soil microbes utilize organic substrates to develop their bodies (assimilation) and maintain their biotic metabolization (dissimilation) for survival (Dijkstra et al., 2008; Coyle et al., 2009; Makarov et al., 2015; Wang et al., 2021). Especially, the abundances of stable C and nitrogen (N) isotopes (i.e., $\delta^{13}C$ and $\delta^{15}N$) in microbial biomass can be used to characterize the properties of the microbial utilization of SOM under different substrate availability conditions (Dijkstra et al., 2008; Coyle et al., 2009; Makarov et al., 2015; Wang et al., 2021). For example, isotope abundances in microbial biomass can represent the metabolized levels of microbially utilized substrates because both C and N in the substrate organic matter are expected to be enriched in heavier isotopes (i.e., ^{13}C and ^{15}N) during microbial metabolization, stimulating the loss of lighter isotopes (i.e., ^{12}C and ^{14}N) from the ecosystem (Dijkstra et al., 2008; Coyle et al., 2009; Craine et al., 2015; Makarov et al., 2015; Wang et al., 2021). If the interested ecosystem has experienced vegetation succession from C4 plants producing ^{13}C-enriched organic matter to C3 plants producing ^{13}C-depleted organic matter (Hobbie and Werner, 2004), ^{13}C abundances in microbial bodies would be a proxy of the age of C substrates (Yoneyama et al., 2001). Another possible implication for isotope abundances in microbial biomass comes from the observation that $\delta^{15}N$ enrichment in the microbial biomass relative to SOM in the surrounding soil environment was negatively correlated with the soil C/N ratio (Dijkstra et al., 2008). This relationship between microbial $\delta^{15}N$ enrichment and the soil C/N ratio indicated that C-limited conditions (i.e., a low C/N ratio) stimulated N dissimilation, resulting in the heavier ^{15}N remaining in microbial bodies while the lighter ^{14}N was depleted. Nitrogen-limited conditions (i.e., a high C/N ratio) stimulated N assimilation, resulting in both ^{14}N and ^{15}N remaining in microbial bodies (Dijkstra et al., 2008; Coyle et al., 2009; Makarov et al., 2015; Wang et al., 2021).

However, the analysis of microbial bodies is not generally applicable to air-dried soil samples from the major soil forms in long-term storage worldwide (Kaiser et al., 2015). Conversely, obtaining information about the microbial utilization of SOM from air-dried soil samples could provide the opportunity to achieve sufficient resolution and spatial coverage of soil analysis data. This would then be practically useful for predicting the C substrates used in soils through microbial decomposition.

Water-extractable organic matter (WEOM) from air-dried soil samples could be used to investigate the microbial activities and processes of SOM decomposition because WEOM is likely derived from microbial cells (Marumoto et al., 1977, 1982; Marumoto, 1984; Unger et al., 2010, 2012; Kaiser et al., 2015). According to Unger et al. (2010, 2012), the rewetting of dry soil releases ^{13}C-enriched CO_2 from soils, demonstrating the stimulation of the decomposition of microbially derived ^{13}C-enriched C substrates. In Japanese paddy field soils, the mineralized C and N observed after the rewetting of air-dried soils are likely derived from microbial cells (Marumoto et al., 1977, 1982; Marumoto, 1984). Given that air-dried soils are the major storage form of soil samples and are well archived by researchers (Kaiser et al., 2015), analyzing the properties of WEOM from air-dried soil samples would be advantageous in characterizing SOM decomposition, which varies spatially from local to global scales (Li et al., 2019; Tang et al., 2020; Zhang et al., 2020).

In this study, we analyzed stable isotope abundances in WEOM from 40 air-dried soil samples gathered from the surfaces of a temperate broadleaved forest in Japan that had experienced a vegetation shift from C4 grassland to C3 tree vegetation. These soils were originally sampled to investigate the environmental distribution of radioactive cesium that was affected by the Fukushima nuclear accident (Atarashi-Andoh et al., 2021) and have been stored in air-dried conditions. The soils were heterogeneously affected by volcanic ash during their development, with various physiochemical and mineralogical properties and C dynamics within a single water catchment (Suzuki, 2002; Nagano et al., 2019). Assuming that WEOM from air-dried soils was derived from soil microbial bodies (Marumoto et al., 1977, 1982; Marumoto, 1984; Unger et al., 2010, 2012; Kaiser et al., 2015), we expected that the enrichments of ^{13}C and ^{15}N in WEOM relative to the bulk soils would be correlated with the concentrations of organo-mineral complexes and short-range order minerals (non-crystalline oxyhydroxides of aluminum and iron, allophane, imogolite, and allophane-like constituents; Imaya et al., 2007), which likely control the SOM stability and thus their availability to soil microbes (Johnson et al., 1995; Baldock and Skjemstad, 2000; Asano and Wagai, 2014; Takahashi and Dahlgren, 2016; Rasmussen et al., 2018; Wagai et al., 2018). We also investigated the similarity between WEOM features and the well-known features of microbial biomass, such as their narrow C/N ratio range being near 10 due to the significant N demand of bacteria (Strickland and Rousk, 2010), a low contribution to bulk SOM (e.g.,1.2% as average Inubushi et al., 2005; Xu et al., 2013), and higher $\delta^{15}N$ than bulk soil (Dijkstra et al., 2008; Coyle et al., 2009; Makarov et al., 2015; Wang et al., 2021) to confirm the possibility of WEOM deriving from microbial biomass.

2. Materials and methods

2.1. Study site and soil sampling

The study forest was a deciduous broadleaved forest in the southern part of the Abukuma Mountains, Ibaraki, Japan (Figure 1). The dominant trees were *Fagus crenata*, *Fagus japonica*, and *Quercus serrata* (C3 vegetation). This forest is an old secondary forest that has not received significant disturbance since the Meiji era (ca. 150 years ago). Some surface soils in this forest have Andic properties, which implies a significant contribution of volcanic ash to the parent material of the soils. Before the Meiji era, *Poaceae* grasslands (C4 vegetation) dominated this area at a time when many artificial fires were set for agricultural purposes (Suzuki, 2002). The mean annual temperature and precipitation were 11°C and 1,900 mm, respectively (Mizoguchi et al., 2002). The soils in the region have been affected by volcanic ash, mainly from

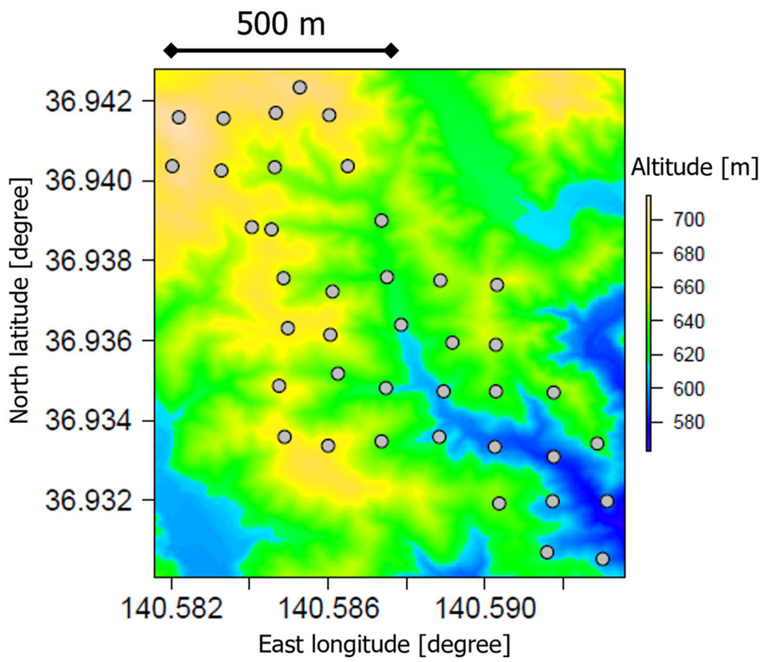

FIGURE 1
A map of soil-sampling locations (dots) in a deciduous broad-leaved forest in Ibaraki, Japan. The colors of the map represent the elevations visualized from a digital elevation model publicly distributed by the Geospatial Information Authority of Japan (http://www.gsi.go.jp/kiban/index.html).

the Abukuma Mountains, which extends over a large area of the northern part of the forest. The soil characteristics in this area are highly heterogeneous in terms of specific landforms and amounts of volcanic ash. Detailed information related to the study forest is available in the studies by Mizoguchi et al. (2002), Yoshinaga et al. (2002), and Suzuki (2002) and chapters in a book by Nakashizuka and Matsumoto (2002).

In August and September 2013, soil samples were collected from a depth of 0–6 cm at 40 locations in the forest, originally to investigate the environmental distribution of radioactive cesium affected by the Fukushima nuclear accident (Figure 1; Atarashi-Andoh et al., 2021). Each sampling point was roughly located in the center of a rectangular grid with a length of 150 m north-south and 120 m east-west. A cylindrical soil core sampler was used to collect soil after removing the organic litter layers (Koarashi et al., 2016). The collected soil samples were gently passed through a 2-mm sieve to remove gravel and plant tissues, followed by fine root removal using tweezers. These soil samples were air-dried to a constant weight at room temperature (ca. 20°C) and were used for analysis.

2.2. Soil analysis

Organic matter extracted from air-dried soil by water (i.e., WEOM from air-dried soil) was analyzed to determine the C and N concentrations, C/N ratio, δ^{13}C, and δ^{15}N. The organic matter was extracted from a 1-g soil sample and 20 mL of deionized water by horizontal shaking for 1 h at 120 rpm. A membrane filter (0.45 µm pore size, HAWP04700, Merck Millipore) equipped with a filter unit (Thermo Scientific 300–4,050, Thermo Fisher Scientific) was used to remove solid materials in the solution. The C concentration in the extracted solution was determined using a total organic C analyzer (TOC-L, Shimadzu). The solution was freeze-dried into a powder. The powder was placed in a tin cup, and their N concentration, C/N ratio, δ^{13}C, and δ^{15}N were determined using an isotope ratio mass spectrometer (IsoPrime100, Isoprime Ltd.) connected to an elemental analyzer (Vario PYRO cube, Elementar). Using these data and the soil and water mixing ratio, we calculated the C and N concentrations, δ^{13}C, and δ^{15}N of WEOM.

From the bulk soil samples, in addition to WEOM, we also measured the C/N ratio, δ^{13}C, δ^{15}N, and concentrations of organo-mineral complexes and short-range order minerals. The C and N concentrations, δ^{13}C, and δ^{15}N were determined using the same instruments as described above. The concentrations of organo-mineral complexes and short-range order minerals were expressed as the sum of aluminum (Al) and half-weighted iron (Fe) selectively dissolved with 2.0 M acid oxalate ammonium (Alo+1/2Feo) (Asano and Wagai, 2014; Takahashi and Dahlgren, 2016; Rasmussen et al., 2018; Wagai et al., 2018). The mineral extraction with 2.0 M acid oxalate ammonium was conducted for 4 h with horizontal shaking at 140 rpm in the dark. The supernatant of the extracted solution after centrifugation at 3,000 G for 10 min was filtered using a centrifugal filter unit (0.1 µm pore size, UFC30VV, Merck Millipore) and analyzed using inductively coupled plasma–optical emission spectrometry (ICP-OES: 5110, Agilent) to measure the selectively dissolved mineral concentrations. We also determined the concentrations of Al and half-weighted Fe dissolved with 0.1 M pyrophosphate sodium solution (Alp+1/2Fep) as minerals specifically associated with organo-mineral complexes. The extraction with a 0.1-M pyrophosphate sodium solution was conducted for 16 h. After centrifugation at 3,000 G for 10 min, the supernatant was centrifugated again at 30,000 G for 60 min. Then, centrifugal filtration and ICP-OES measurements were conducted. The difference between Alo+1/2Feo and Alp+1/2Fep (here represented as Alo-p+1/2Feo-p) represented the concentration of short-range order minerals (Courchesne and Turmel, 2008). The enrichments of δ^{13}C and δ^{15}N in WEOM were calculated as the differences in the δ^{13}C and δ^{15}N values between WEOM and bulk soil samples. The results of the soil analysis are presented in Supplementary Tables S1–S3 in Supplementary Information.

2.3. Data analysis

The data were analyzed with the R software 4.1.1 (R Core Team, 2021). The correlations among metrics were statistically evaluated using the *cor.test* function. Then, these correlations were compared between WEOM and bulk soil samples from the viewpoint of whether there were any apparent differences in correlation directions (i.e., positive, negative, or insignificant). Statistical differences in metrics between WEOM and bulk soils were determined using a *t*-test by the *t.test* function, and a *p*-value of < 0.05 was considered statistically significant.

3. Results

The C and N concentrations, δ^{13}C, and δ^{15}N showed positive correlations between WEOM and bulk soils, but the C/N ratio did not (Figure 2). The C and N concentrations in bulk soils were 46–230 mg C g^{-1} dry soil and 2–14 mg N g^{-1} dry soil, respectively; whereas, the corresponding values in WEOM were <3.6% of those in bulk soils (1.0% and 1.9% as averages for C and N, respectively). The correlation coefficients (r) for the C and N concentrations between WEOM and bulk soils were 0.80 or higher ($p < 0.01$). The δ^{13}C and δ^{15}N values ranged from −27.5 to −21.3‰ and 1.2 to 5.0‰ in bulk soils, respectively, while these values ranged from −26.7 to −23.0‰ and 3.5 to 9.2‰ in WEOM, respectively. These isotope abundances were significantly higher in WEOM than in bulk soils ($p < 0.01$), showing significant correlations between WEOM and bulk soils ($r \geq 0.59$, $p < 0.01$). However, for the C/N ratio, there was a non-significant correlation between WEOM and bulk soils ($r = -0.06$, $p = 0.73$), showing narrow ranges of values around 10 in the WEOM. The C/N ratio in WEOM, ranging from 7.9 to 11.9, was significantly lower than that in bulk soil samples (13.9–25.6) ($p < 0.05$).

Positive correlations were also found between most of the C and N metrics (in both bulk soil and WEOM) and selectively dissolved mineral concentrations (Alo+1/2Feo, Alp+1/2Fep, and Alo-p+1/2Feo-p) (Table 1). The ranges of Alo+1/2Feo, Alp+1/2Fep, and Alo-p+1/2Feo-p were 3.7–27.2, 2.0–16.3, and 1.0–12.0 mg g^{-1} dry soil, respectively (Supplementary Table S3). The correlation coefficients for the relationship between these minerals and the C and N concentrations, δ^{13}C, and δ^{15}N in the two fractions were 0.46 or greater ($p < 0.01$), except for the non-significant correlations of the WEOM C and N concentrations with Alo-p+1/2Feo-p ($r = 0.09$ to 0.14, $p > 0.05$).

Conversely, the relationships between the stable isotope enrichments in WEOM and the concentrations of selectively dissolved minerals showed the opposite trend between ^{13}C and ^{15}N (Figure 3). The δ^{13}C enrichments were negatively correlated with Alo+1/2Feo ($r = -0.66$, $p < 0.01$), whereas the δ^{15}N enrichments were positively correlated with Alo+1/2Feo ($r = 0.51$, $p < 0.01$). Similarly, there were negative correlations for Alp+1/2Fep and Alo-p+1/2Feo-p with the δ^{13}C enrichments ($r < -0.62$, $p < 0.01$) but positive correlations with the δ^{15}N enrichments ($r > 0.51$, $p < 0.01$).

4. Discussion

As we expected, the lower variation in C/N ratio (values around 10) was the feature of WEOM from air-dried soil, rather than that of bulk SOM (Figure 2). This WEOM feature partially supports the significant potential that the WEOM was derived from microbial cells. This suggestion especially relies on the well-known features of microbial ecology, in which microbial communities, particularly bacterial communities dominating in surface mineral soils, have bodies with a C/N ratio near 10 due to the significant N demand of bacteria (Strickland and Rousk, 2010). Additionally, <3.6% of the contribution of WEOM to bulk SOM is of the same magnitude as the globally estimated contributions (1.2% as an average) of microbial biomass C to bulk soil organic C (Inubushi et al., 2005; Xu et al., 2013). A possible implication of the observed higher δ^{15}N in WEOM (3.5–9.2‰) than in bulk soils (1.2–5.0‰) (Figure 2) is the isotope fractionation of N resulting from the metabolization of N substrates (Dijkstra et al., 2008; Coyle et al., 2009; Makarov et al., 2015; Wang et al., 2021), supporting our inference on the origins of WEOM from air-dried soils. While there are other possible organic origins of WEOM, such as fine plant debris and root exudates, the suggested possibility of significant linkage between WEOM and soil microbial bodies enables us to expect a significant linkage of variation in stable isotope enrichments of WEOM (Figure 3) to the microbial utilization of soil C and N substrates, as described below.

A possible implication for the observed variations in δ^{13}C and δ^{15}N enrichments of WEOM along with selectively dissolved mineral concentrations (Figure 3) is that the age and metabolized levels of SOM utilized by soil microbes depend on the selectively dissolved mineral concentrations (Figure 4), which likely control SOM stability (and thus availability) (Shirato et al., 2004; Asano and Wagai, 2014; Takahashi and Dahlgren, 2016; Rasmussen et al., 2018; Wagai et al., 2018). The age of utilized substrates determining the variation in δ^{13}C of substrate organic matter and microbial bodies (Yoneyama et al., 2001) is likely reliable in soils with a succession history from C4 to C3 vegetation, such as the investigated forest and many other Japanese forests (Yoneyama et al., 2001; Suzuki, 2002; Hiradate et al., 2004; Takahashi and Dahlgren, 2016), where the δ^{13}C values of photosynthesized organic matter apparently differ between the two plant types (∼14‰ for C4 and −27‰ for C3; Hobbie and Werner, 2004). No evidence of vegetation succession fluctuating the substrate δ^{15}N was available in the study forest. Thus, if microbial bodies are the major sources of WEOM derived from air-dried soils, the δ^{13}C and δ^{15}N enrichments in WEOM can

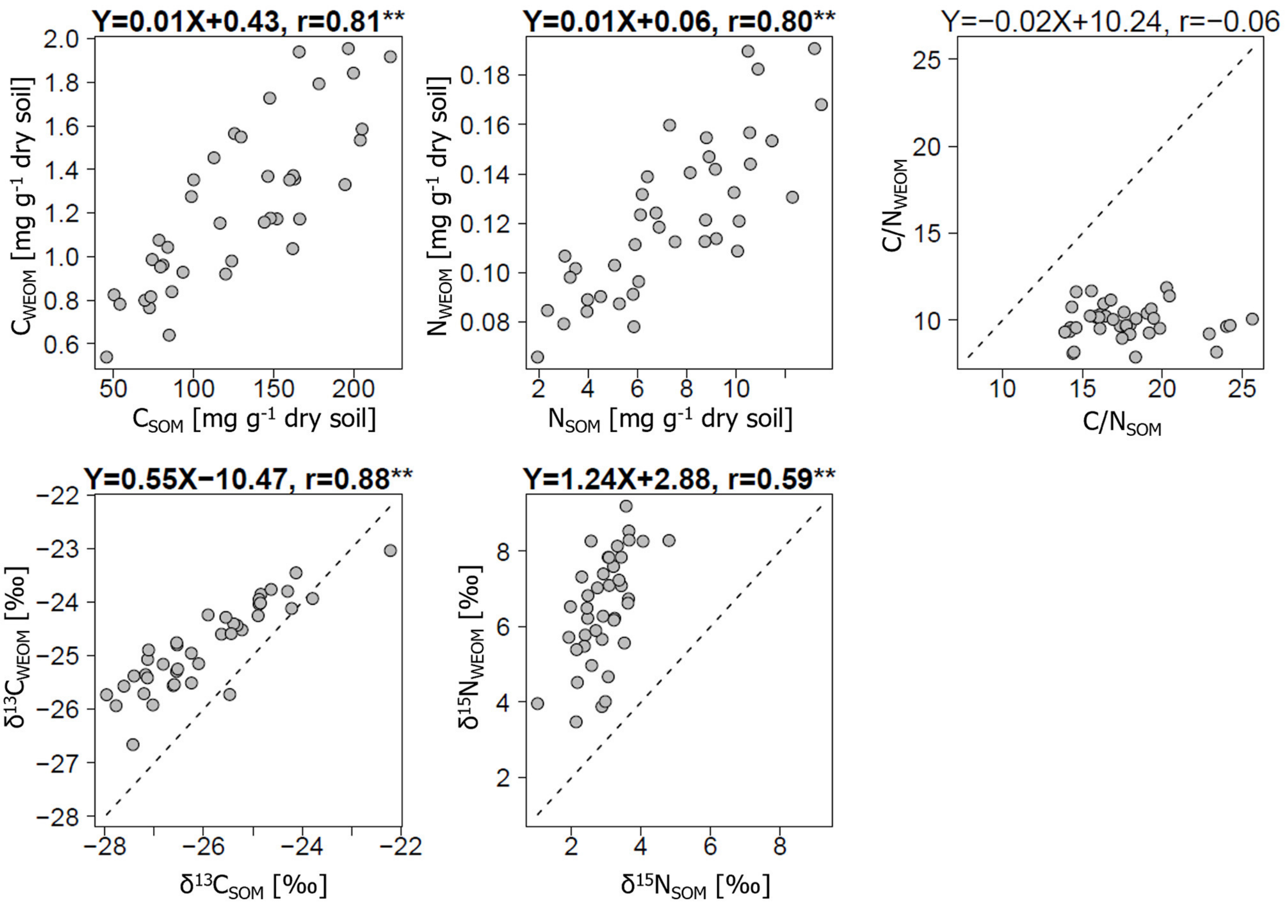

FIGURE 2
Relationships of carbon (C) and nitrogen (N) metrics [C and N contents, C/N ratio, and stable C and N isotope abundances (δ^{13}C, δ^{15}N)] between bulk soils and water-extractable organic matter (WEOM) from air-dried soils. All metrics were significantly different between the bulk soils and WEOM at a p-value of < 0.01. Linear regression equations are presented with correlation coefficients (r), suggesting their statistical significance by $^*p < 0.05$ and $^{**}p < 0.01$.

TABLE 1 Correlation coefficient $^{a)}$ between C and N metrics and selectively dissolved mineral concentrations $^{b)}$.

Metric	Bulk soil or WEOM	VS Al_o+1/2Fe_o	VS Al_p+1/2Fe_p	VS Al_{o-p}+1/2Fe_{o-p}
C concentration	Bulk soil	**0.70****	**0.78****	**0.48****
	WEOM	**0.32***	**0.46****	0.09
N concentration	Bulk soil	**0.68****	**0.75****	**0.46****
	WEOM	**0.37***	**0.50****	0.14
δ^{13}C	Bulk soil	**0.66****	**0.66****	**0.55****
	WEOM	**0.61****	**0.66****	**0.43****
δ^{15}N	Bulk soil	**0.43****	**0.35***	**0.46****
	WEOM	**0.70***	**0.65****	**0.64****

[a)] Bold values with * and ** represent significant coefficients at a p-value of < 0.05 and < 0.01, respectively.

[b)] Selectively dissolved mineral concentrations were the concentrations of aluminum (Al) and half-weighted iron (Fe) dissolved by 2.0 M acid oxalate ammonium (Alo+1/2Feo), 0.1 M pyrophosphate sodium (Alp+1/2Fep), and the two dissolving solutions (Alo-p+1/2Feo-p).

be used to deduce the age and metabolized levels of the substrate organic matter utilized by soil microbes, respectively (Figure 4). This analysis takes into account the heterogeneous distribution of microbially available substrates (Koarashi et al., 2009; Mueller et al., 2012). Under this statement, microbes in soils with large concentrations of organo-mineral complexes and short-range order minerals, resulting in a low availability of organic substrates, are likely to incorporate relatively young (added by C3 vegetation since the Meiji era) but well-metabolized organic matter, which has been depleted in ^{13}C but enriched in ^{15}N compared to bulk soils. In other

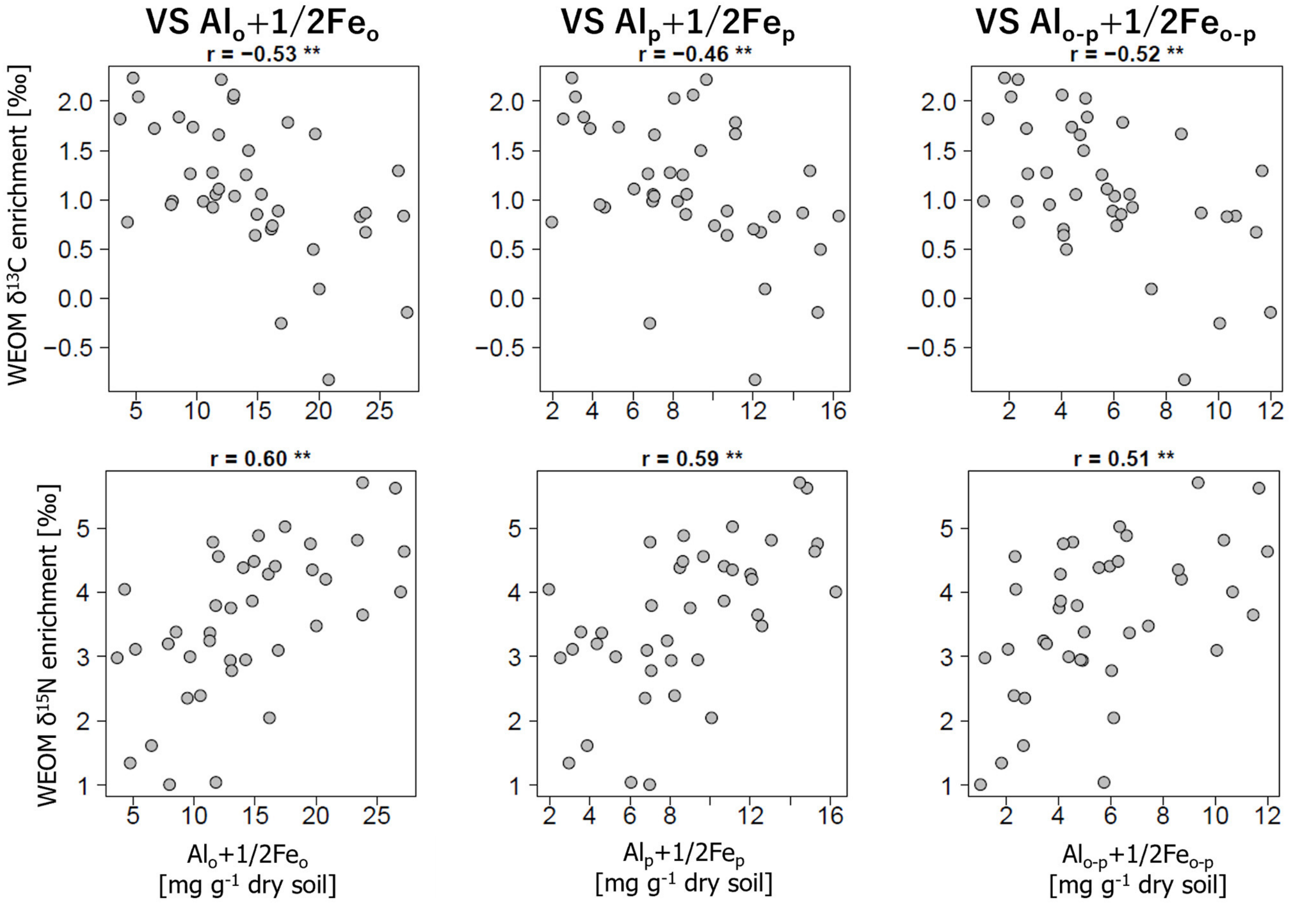

FIGURE 3

Relationships of the δ^{13}C and δ^{15}N enrichment in WEOM compared to bulk soils with the concentrations of Alo+1/2Feo (left panels), Alp+1/2Fep (center panels), and Alo-p+1/2Feo-p (right panels). Correlation coefficients (r) are presented above the panels with their statistical significance ($^{*}p$ < 0.05, $^{**}p$ < 0.01).

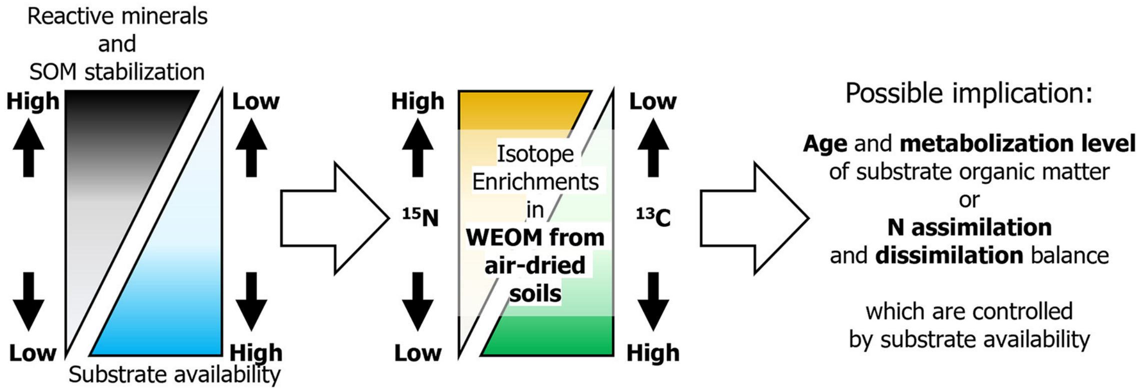

FIGURE 4

Proposed relationships among selectively dissolved minerals, SOM stabilization, substrate C availability, microbial ^{13}C and ^{15}N enrichments, and microbial SOM utilization (i.e., the C and N assimilation and dissimilation, and the ages and metabolized levels of utilized SOM) in forest soils that had experienced vegetation changes from C4 to C3 plants since the Meiji era (ca. 150 years ago). Here, we designated the ^{13}C depleted SOM added by the C3 vegetation since the Meiji era as young.

soils with lower concentrations of organo-mineral complexes and short-range order minerals, and consequently a high availability of organic substrates, microbes likely incorporate older (added by C4 vegetation before the Meiji era) but less-metabolized organic matter, which is enriched in ^{13}C but not in ^{15}N compared to bulk soils.

Another implication is possible as substrate availability controls the balance of microbial N assimilation and dissimilation (Figure 4; Dijkstra et al., 2008; Coyle et al., 2009; Wang et al., 2021), with particular emphasis on the positive correlation between the $\delta^{15}N$ enrichments in WEOM and selectively dissolved mineral concentrations (Figure 3). Specifically, in soils where C availability is relatively limited compared to N availability, microbes are likely to promote nitrogen dissimilation, resulting in the retention of ^{15}N while depleting ^{14}N in their bodies (Dijkstra et al., 2008). However, in soils with a relatively limited N availability compared to C availability, microbes stimulate N assimilation, resulting in ^{15}N-depleted bodies (Dijkstra et al., 2008). In a different vein, substrate availability-controlling microbial assimilation and dissimilation have been presumed with soil and substrate C/N ratio (Dijkstra et al., 2008; Coyle et al., 2009; Wang et al., 2021). The negligible relationship between the $\delta^{15}N$ enrichment in WEOM and the soil C/N ratio in the investigated soils ($r = -0.23$, $p > 0.05$; Supplementary Figure S1) indicated that the selectively dissolved mineral concentration rather than soil C/N ratio is a better index for substrate availability for soil microbes in these soils.

Regardless of which implication is a representative of the relationship between microbial stable isotope enrichments and selectively dissolved mineral concentrations in the study forest, characterizing the properties of substrate organic matter for soil microbes and their metabolization processes are the keys to reliably inferring the microbial decomposition of SOM and the consequent CO_2 release from soils under changing environments. For example, well-metabolized organic matter is sometimes recalcitrant to microbial decomposition (Six et al., 2002; Lützow et al., 2006; Wagai et al., 2018), and its decomposition is expected to be sensitive to temperature, according to the Arrhenius theory, which describes the response of C release from the soil through microbial SOM decomposition to increasing temperature (Davidson and Janssens, 2006). The relationships between the age of SOM and its decomposability are also a global concern for predicting the future of the global C cycle (Lawrence et al., 2020). Thus, the $\delta^{13}C$ and $\delta^{15}N$ signatures, particularly their enrichments in WEOM relative to bulk soils, which can be obtained from air-dried soils, have a significant potential to obtain a better understanding for microbial C and N utilization in soils.

5. Conclusions

Our findings indicate that WEOM obtained from air-dried soil exhibiting relatively consistent C/N ratios close to a value of 10 is present in relatively small amounts in soils, and displays more enriched $\delta^{13}C$ and $\delta^{15}N$ values compared to bulk SOM. These findings suggest that the WEOM is likely derived from microbial biomass. However, it is possible to note that the possibility of other organic sources, such as fine plant debris and root exudates, also requires further investigation. Assuming the WEOM consists of microbial biomass, the observed variations in $\delta^{13}C$ and $\delta^{15}N$ enrichments of the WEOM and selectively dissolved mineral concentrations indicated a significant linkage between microbial substrate utilization and substrate availability. Thus, $\delta^{13}C$ and $\delta^{15}N$ enrichments in WEOM from air-dried soils have significant potential for investigating the microbial utilization of substrate organic matter in a Japanese temperate broadleaved forest. Since this method is easily applicable to air-dried soils, the primary storage form of soils for researchers, analyzing stable isotope abundances of WEOM from air-dried soils would have a significant advantage in determining SOM decomposition and decomposability, which vary substantially from local to global scales. It should be noted that the findings of the present study are based on the insufficient types of soil collected at one site and the correlation analysis between metrics. Therefore, further study is required to address the generality of the findings from this study.

Data availability statement

The primary datasets supporting this study's findings are available in Supplementary Tables S1–S3. Additional data and R scripts for data analysis are also available from the authors upon reasonable request.

Author contributions

HN established the basic research design, conducted all analyses, including software preparation, validation, visualization, and wrote the manuscript. MA-A and JK conducted the soil sampling. MA-A, ST, TY, NK, and JK contributed to the detailed research design, soil analysis, data validation, interpretation of the results, and editing of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/ffgc.2023.1228053/full#supplementary-material

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