Biology and Fertility of Soils (2019) 55:825–832
https://doi.org/10.1007/s00374-019-01374-7

SHORT COMMUNICATION

Effect of sieving and sample storage on soil respiration and its temperature sensitivity (Q10) in mineral soils from Germany

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Received: 20 September 2018 / Revised: 5 June 2019 / Accepted: 17 June 2019 / Published online: 26 June 2019

Abstract

Knowledge about spatial patterns of soil respiration (SR) and its temperature sensitivity (Q10) is of emerging relevance for assessing carbon fluxes across the landscape. Related experiments are often conducted under controlled laboratory conditions and usually rely on soil samples, which are sieved and stored. Here, we investigated the effect of sieving and storage on SR and Q10. We took 14 samples from different land use types and soil textures. Samples were sieved to 2 mm at field-moist conditions and split into four treatments: sieved/no-storage, sieved/freeze-storage (−18 °C), sieved/cold-storage (+4 °C), and sieved/dry-storage (+40 °C). The storage time was 7 weeks. Intact soil cores were used as a control. The SR was not significantly affected by sieving/no-storage, sieving/freeze-storage, and sieving/cold-storage compared with the control. Yet, sieving/dry-storage significantly increased SR but all samples were similarly affected ($r = 0.81$ for the correlation between SR after sieving/dry-storage and SR in the control). The Q10 of sieving/no-storage (1.94 ± 0.28), sieving/freeze-storage (1.94 ± 0.23), sieving/cold-storage (2.37 ± 0.29), and sieving/dry-storage (2.29 ± 1.35) did not differ significantly from the control (2.12 ± 0.23). All samples responded similar to sieving and storage ($r = 0.68$–0.73 for the correlation between Q10 in each respective treatment and Q10 in the control), with the exception of sieved/dry-storage ($r = 0.09$). We conclude that sieving at field-moist conditions and subsequent freeze- or cold-storage is acceptable to derive SR and Q10 for the here reported storage time. Although dry-storage may be acceptable for the comparison of SR between samples, it should be avoided for realistic estimates of SR and for the determination of Q10.

Keywords Heterotrophic soil respiration · Carbon mineralization · Soil pretreatment · Drying and rewetting

Introduction

Heterotrophic soil respiration (SR) is a major component of the global carbon (C) cycle and an accurate assessment of its spatiotemporal patterns is of immense importance for predicting C fluxes. One of the major regulating factors of SR is temperature. The relation between SR and temperature is commonly expressed as Q10 value, which is the increase of SR by a 10 °C rise in temperature (Kirschbaum 1995; Van 't Hoff 1898). While the Q10 value is commonly implemented as a fixed value of 1.5 or 2 in modeling approaches (Foereid et al. 2014; Potter et al. 1993; Raich et al. 1991), there is consensus that Q10 can be highly variable and might range from 1 to higher than 12 (Hamdi et al. 2013). Many studies have been conducted to explore spatiotemporal patterns of SR and Q10 values across the landscape (e.g., Fierer et al. 2006; Wang et al. 2016). Many of these studies were conducted under controlled laboratory conditions in order to vary the factor of interest (e.g., temperature) while keeping interfering factors constant (e.g., Fierer et al. 2006; Lefèvre et al. 2014; Wang et al. 2016). In this regard, Kirschbaum (2006) considered that "laboratory incubations would provide the best and least biased, basis for estimating the temperature dependence of organic matter decomposition." Other researchers, however, questioned the transferability of controlled laboratory experiments to real-world conditions (Černohlávková et al. 2009; Herbst et al. 2016; Lamparter et al. 2009; Lomander

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00374-019-01374-7) contains supplementary material, which is available to authorized users.

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et al. 1998). The authors criticized that sample preprocessing like sieving and storage might alter microbial biomass, microbial activity, microbial community composition, and the availability of substrate. However, storage is mostly inevitable for practical reasons (e.g., Gritsch et al. 2015; Lefèvre et al. 2014; Meyer et al. 2017) and sieving (mostly to < 2 mm) is a common practice to remove roots, plant residues, and rock fragments and to ensure homogenization of the soil (e.g., Conant et al. 2008; Fierer et al. 2006; Wang et al. 2016). Only few studies on Q10 were conducted using undisturbed and/or fresh samples (e.g., Miller and Geisseler 2018; Reichstein et al. 2005)

An alteration of SR and Q10 by sieving and storage might be straightforward if all soils were similarly affected. In this case, a correction factor could be applied to derive the real-world values. However, Hassink (1992) reported that sieving has larger effects in loamy than in sandy soils and Lee et al. (2007) reported that soils with high organic carbon (SOC) contents are more susceptible to storage than soils with low SOC contents. Hence, sieving and storage might not only induce an offset in SR and Q10 estimates but might also lead to false conclusions about relations and comparisons among samples and, hence, to false conclusions about spatial patterns. Several studies have been conducted to investigate the effect of sieving (e.g., Datta et al. 2014; Hassink 1992; Juarez et al. 2013; Thomson et al. 2010) or storage (Černohlávková et al. 2009; Stenberg et al. 1998; Zelles et al. 1991) on SR. One major drawback of previous studies is that SR, which was measured after various preprocessing treatments, has often not been compared with an undisturbed and non-stored control, which might represent the "real-world" conditions (e.g., Černohlávková et al. 2009; Datta et al. 2014; Stenberg et al. 1998; Zelles et al. 1991). Further, the effect of sieving and storage on the temperature sensitivity of SR (Q10) has, to our knowledge, not been investigated yet.

The aim of this study was to investigate the effect of sieving and storage on SR and Q10. We hypothesized that (1) sieving and storage have a significant effect on SR and Q10 and that (2) soils that vary in texture and SOC contents respond with different extent to sieving and storage, which complicates comparisons between samples and interpretations of spatial patterns. To investigate these hypotheses, we took 14 soil samples including different land use types and textures in North Rhine-Westphalia, Germany. Samples were sieved at field-moist conditions and split into four treatments: no storage, freeze-storage, cold-storage, and dry-storage. Samples were stored for 7 weeks. Intact soil cores, which were neither sieved nor stored, were used as a control.

Material and methods

We sampled 14 sites in North Rhine-Westphalia (Germany), including 5 cropland sites, 4 grassland sites, and 5 forest sites, showing a broad range of soil textures and SOC contents (7–96% sand, 1–33% clay, 1.1–4.8% SOC, for details see Table S1). At each site, 20 soil cylinders of 100 cm^3 were taken from the A horizon (1–5 cm depth). Five soil cylinders remained intact, which were put into polyethylene incubation vessels immediately after sampling ("control" = not sieved, not stored). Soil contained within the remaining soil cylinders was combined to a composite sample and subsequently sieved to < 2 mm at field-moist conditions (within 24 h after sampling). Parts of each composite sample were homogenized using a kitchen hand mixer. Subsequently, 80 g of moist soil was filled into incubation vessels (sieved/non-stored, Table 1), three replications each, and slightly recompressed (following Breulmann et al. 2014). Mixing was conducted because in many studies it represents a common practice to homogenize the soil (Meyer et al. 2017; Smith 2005). The incubation vessels containing soil of the control and of the sieved/non-stored treatment were subsequently pre-incubated at field-moist conditions for 6 days at 4 °C. Thereafter, they were placed into a Respicond system for respiration measurements (see below).

The remaining soil of each sieved composite sample was split into three parts and stored for 7 weeks (a) at 4 °C in a cold room (sieved/cold-storage, Table 1), (b) at −18 °C in a freezer (sieved, freeze-storage), or (c) were dried at 40 °C and then stored for 7 weeks at room temperature (sieved/dry-storage). After 7 weeks, the samples of the stored treatments were thawed at 4 °C (sieved/freeze-storage treatment) or rewetted to the original soil moisture level of the intact control (sieved/dry-storage treatment). Samples were homogenized with a kitchen hand mixer. Subsequently, 80 g moist soil was filled into incubation vessels, three analytical replications each, and slightly recompressed. Like the non-stored treatments, they were pre-incubated at 4 °C for 6 days and subsequently placed into the Respicond system for respiration measurements (see below).

Soil respiration measurements were conducted using an automated respirometer that allows incubating 95 samples in parallel (Respicond VIII, Nordgren Innovations AB, Sweden). The system provides a continuous measurement of CO_2 evolution by trapping CO_2 in potassium hydroxide (KOH) (Nordgren 1988). The decrease in electrical conductivity in KOH solution caused by CO_2 entrapment was automatically measured every hour by platinum electrodes. The changes in conductivity were automatically transformed to CO_2 evolution rates, based on Eq. 1 where A is a conductivity constant that depends on the molarity of the KOH solution, C_{t0} is the conductance of the fresh KOH measured at the beginning of the incubation time, and C_{t1} is the conductance at time t. We are aware that autotrophic bacteria can take up parts of the produced CO_2, which may lead to underestimations of soil respiration and, as temperature dependent process, may induce a bias in Q10 estimates. Yet, as measured CO_2 production is considerably larger than rates of CO_2

Table 1 Details of the treatments and mean values and standard deviation of soil respiration rates at 25 °C (SR_{25}) and Q10 values. In each column, values with different letters are significantly different (p<0.05)

Treatment	Sieving	Storage time	Storage temperature	Independent samples	Replicates per sample	SR_{25} (μg CO_2 h^{-1} g^{-1} soil)	Q10 value
Control	No	No	–	14	5	3.04 ± 1.64^{a}	2.20 ± 0.23^{ab}
Sieved/non-stored	Yes	No	–	14	3	3.38 ± 1.62^{a}	1.94 ± 0.28^{b}
Sieved/freeze-storage	Yes	7 weeks	− 18 °C	14	3	3.57 ± 1.71^{a}	1.94 ± 0.23^{b}
Sieved/cold-storage	Yes	7 weeks	4 °C	14	3	2.57 ± 1.15^{a}	2.37 ± 0.29^{a}
Sieved/dry-storage	Yes	7 weeks	40 °C	14	3	6.46 ± 3.07^{b}	1.94 ± 0.40^{b}

fixation reported by Xiao et al. (2018), we assume this process to have a negligible influence on the outcome.

$$CO_2 = A \times \frac{C_{t0} - C_{t1}}{C_{t0}} \quad (1)$$

After pre-incubation at 4 °C for 6 days, soil samples were sequentially set to 5 °C, 10 °C, 15 °C, 20 °C, and 25 °C by controlling the temperature of the water bath of the Respicond system. Samples were kept at each temperature for 24 h (see also Gritsch et al. 2015). The first 12 h after each temperature rise were treated as equilibration time. This was necessary because soil microorganisms may need a couple of hours to adapt to the new temperature level. The subsequent 12 h were used for the calculation of soil respiration. After completion of each temperature level, vessels were left open for about 30 min to equilibrate with ambient O_2 concentrations. The KOH solution was replaced subsequently. The short-term incubation approach was chosen to minimize effects of changing C pool sizes during the incubation. Longer incubation times can underestimate Q10 because SOC decreases with increasing incubation time (Hamdi et al. 2013; Kirschbaum 2006).

Soil respiration was expressed as the average hourly CO_2 release per gram of dry fine soil (< 2 mm). The exact amount of dry fine soil in each incubation vessel may vary as a result of variable soil moisture and particles > 2 mm. Hence, the soil contained within each incubation vessel was dried and, in case of the control, sieved to < 2 mm after completion of the incubation.

An exponential equation was used to calculate the relationship between temperature and soil respiration, which was fitted over the total temperature range of 5–25 °C according to Eq. 2 where SR_T is soil respiration at a given temperature, a and b are fitted parameters, and T is temperature.

$$SR_T = a \times \exp^{b \times T} \quad (2)$$

The Q10 value was then calculated by inserting parameter b into Eq. 3.

$$Q10 = \exp^{10 \times b} \quad (3)$$

The mean value of the 3–5 analytical replicates per sampling site and treatment was used for statistical tests. One sample was excluded from statistical tests because it showed an irregular response to temperature in the sieved/dry-storage treatment. We used one-way ANOVA with sampling site as a block factor to investigate whether Q10 or SR was significantly different across treatments. In case of significant effects ($p < 0.05$), Tukeys HSD post hoc test was applied to investigate the significances between different treatments. Statistics and figures were performed with R (version 3.2.3, R Core Team 2013).

In each treatment, soil was incubated at the original soil moisture level of each respective control sample (Table S1). Hence, soil moisture varied across samples but not across treatments. As the goal of this study was to explore the effect of sieving and storage in comparison with undisturbed soil under non-manipulated conditions, we have to accept this natural variation in water content. Almost all of the samples were within the range of 30–52% of water holding capacity (WHC, see Table S1) and hence, in a range in which neither water deficiency nor oxygen deficiency severely limits SR (Meyer et al. 2018). Thus, we expect no major effect of variable soil moisture contents on soil respiration. Only three samples do not fulfill this criterion (21–28% of WHC). But as excluding these samples does not affect the results, we are confident that the here considered variations in water content have no major effect on the response of SR to sieving and storage.

Results and discussion

Effect of sieving and storage on soil respiration at 25 °C

Sieving the soil at field-moist conditions did not significantly alter soil respiration at 25 °C (SR_{25}) relative to the control (Table 1, Fig. 1a, $p = 0.99$). This finding is in agreement with results from Černohlávková et al. (2009) and Thomson et al. (2010), who also reported that sieving at field-moist conditions did not significantly affect SR. Other studies, in contrast, suggested that sieving may result in a temporary increase of SR as aggregates are disrupted, which releases previously stabilized SOC (Hassink 1992). However, Hassink (1992) assumed that the degree of disruption is larger in dried samples

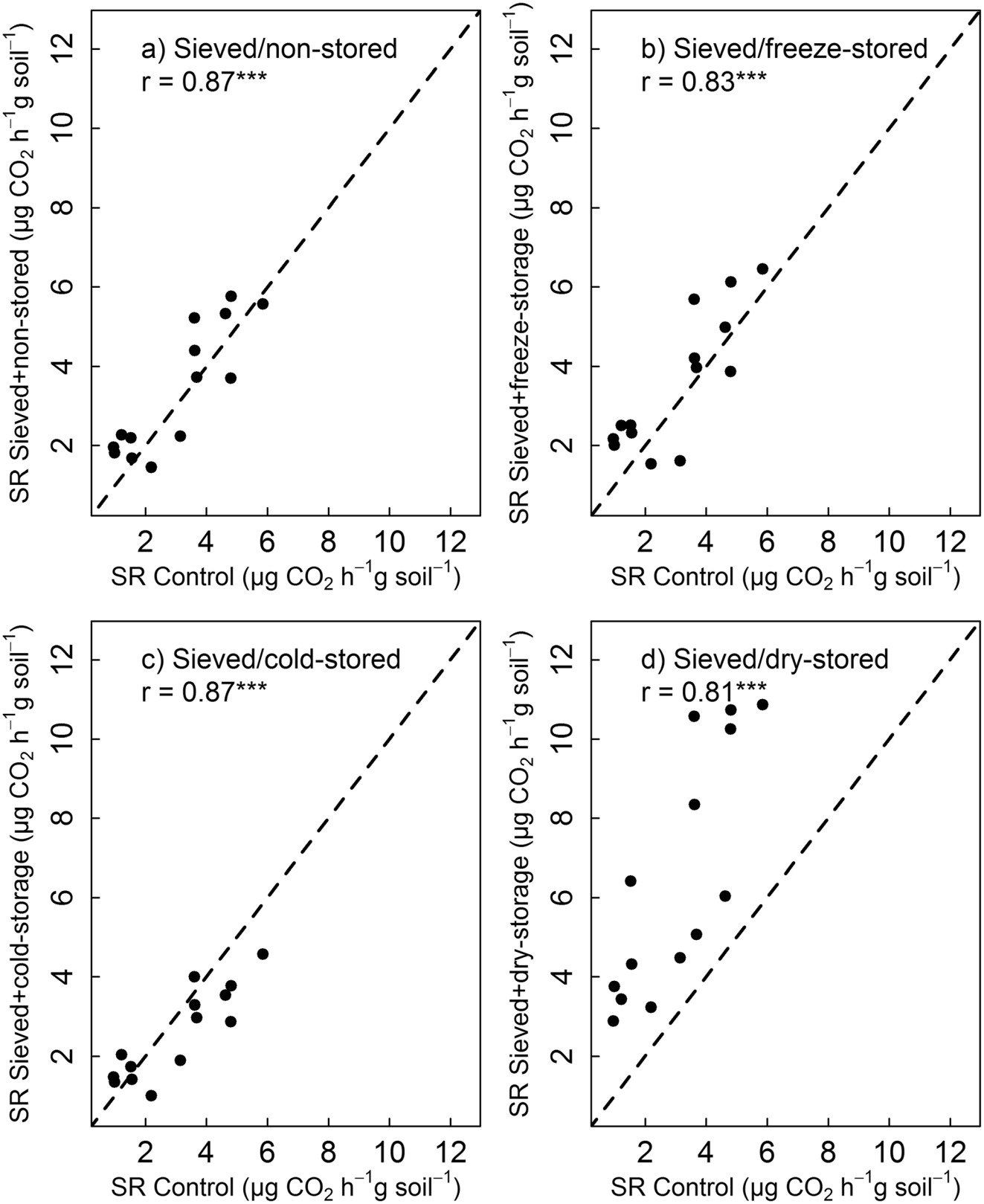

Fig. 1 Relation between SR_{25} measured in the four treatments and SR_{25} measured in the control, **a** for sieved/non-stored soils, **b** for sieved/freeze-stored soils, **c** for sieved/cold-stored soils, **d** for sieved/dry-stored soils

than in moist samples. In line with our results and previous studies, we suggest that sieving at field-moist condition delivers results comparable with undisturbed conditions. Yet, we cannot exclude that sieving of dried soils leads to larger deviations as we did not compare the sieving/dry-storage treatment with dried but not sieved samples.

If soils have to be stored after sieving, cold-storage at 4 °C is generally recommended by ISO 10381-6 (1993). And indeed, we found that sieving/cold-storage had no significant effect on SR_{25}, although a tendency towards a slight reduction of SR_{25} compared with the sieved/non-stored treatment (23% on average, $p = 0.80$, Table 1) and compared with the control (Fig. 1c, $p = 0.97$) was recognizable. A probable reason for this reduction is the finding that soil respiration takes place even at such low temperatures. Indeed, our soil respiration measurements revealed a substantial CO_2 release even at 5 °C (0.87 ± 0.76 μg CO_2 h^{-1} g $soil^{-1}$, not shown). Hence, it is probable that considerable amounts of labile and easily degradable SOC have already been mineralized within the 7 weeks of storage at 4 °C, which might explain the slightly lower SR_{25} after cold-storage. This assumption is supported by results of Stenberg et al. (1998), who reported that soil respiration decreased with increasing duration of the cold-storage period. Although we did not measure the effect of storage duration, this suggests that storage at 4 °C delivers unbiased results only when storage duration is kept short.

While the mineralization of SOC proceeds during storage at 4 °C, this is probably reduced by freeze-storage at − 18 °C. Indeed, freeze-storage had no significant effect on SR_{25} compared with the sieved/non-stored treatment ($p = 0.99$, Table 1) nor compared with the control (Fig. 1b, $p = 0.95$) and there was no reduction of the mean SR_{25} (Table 1). This indicates that freezing largely reduces the mineralization of C during storage and preserves the original amount and availability of SOC.

In contrast to cold-storage and freeze-storage, only dry-storage induced a significant increase of SR_{25} upon rewetting compared with the sieved/non-stored treatment (99% on average, $p < 0.001$, Table 1) and compared with the control (Fig. 1*d*, $p < 0.001$). This outcome is in agreement with previous studies on drying-rewetting effects (Birch 1958; Franzluebbers 1999). The reasons for this so called Birch effect are still debated (Xiang et al. 2008) and might result from microbial stress (Schimel et al. 2007), changes in substrate supply due to aggregate disruption (Denef et al. 2001; Miller et al. 2005), or microbial death during drying with subsequent mineralization of microbial debris upon rewetting (Van Gestel et al. 1993). However, while many studies report that drying-rewetting events cause a short-term increase of SR (e.g., Franzluebbers (1999) reported that the effect lasted for 0–3 days), our results reveal that the effect was significant even after 6 days of pre-incubation at 4 °C and subsequent 5 days of incubation from 5 to 25 °C.

An effect of sample pre-processing (including both sieving and storage) on SR_{25} would be straightforward if it only induces an offset in the measured SR_{25} rate without affecting comparisons between samples. This would allow to apply a correction factor or to study spatial patterns of SR even if values might deviate from undisturbed conditions. Indeed, samples with high SR_{25} in the control revealed also high SR_{25} in the sieved and stored treatments, i.e., the rank order was similar in all treatments (Fig. 1). Thus, all samples responded rather similar to sieving and storage. This opposes Hassink et al. (Hassink 1992), who reported that sieving had larger effects in loamy than in sandy soils and Lee et al. (2007), who reported that soils with high SOC contents were more susceptible to the effects of storage. Our results suggest that each of the conducted treatments might be acceptable for deriving spatial patterns of SR_{25} across the landscape although it has to be kept in mind that values might considerably deviate from undisturbed conditions after dry-storage and subsequent rewetting. Hence, if storage is required after sieving, we propose that freeze-storage or short-term cold-storage represent the best options to preserve both the real-world values and their spatial patterns.

Effect of sieving and storage on Q10

Sieving might disrupt aggregates and release C, which was previously occluded in aggregates (Hassink 1992). In this regard, the C quality-temperature hypothesis predicts that the Q10 value decreases with decreasing stability of SOC, i.e., that labile SOC is less sensitive to temperature changes than stabilized or biochemically recalcitrant SOC (Bosatta and Agren 1999; Conant et al. 2008; Davidson and Janssens 2006). And indeed, Q10 values revealed a tendency to decrease upon sieving and associated aggregate disruption (Table 1, Fig. 2 a) although this effect was not significant ($p = 0.19$). The insignificance of this effect could be explained by the finding that sieving had no major effect on SR_{25} (sieved/not-stored, see above), which indicates that the release of previously stabilized SOC was small.

Among the sieved treatments, there was a considerable effect of storage method. Cold-storage significantly increased the Q10 value compared to the sieved/non-stored treatment (Table 1, $p = 0.004$). Thus, sieving/cold-storage also overestimated Q10 compared to the control (Fig. 2c) although this difference was not significant ($p = 0.54$). As supported by a lower SR_{25} after cold-storage, considerable amounts of labile C might have been mineralized during 7 weeks of cold storage. Hence, SOC might be comparatively enriched in stable and slow-cycling SOC after cold-storage. In line with the C quality-temperature hypothesis, Q10 values are higher for stable and slow-cycling SOC than for fresh and easily degradable SOC (Bosatta and Agren 1999; Conant et al. 2008; Davidson and Janssens 2006). This might explain the higher Q10 values after cold-storage.

In contrast, neither freeze-storage (Table 1, $p = 0.99$) nor dry-storage (Table 1, $p = 0.99$) had a considerable effect on the Q10 value compared with the sieved/non-stored treatment.

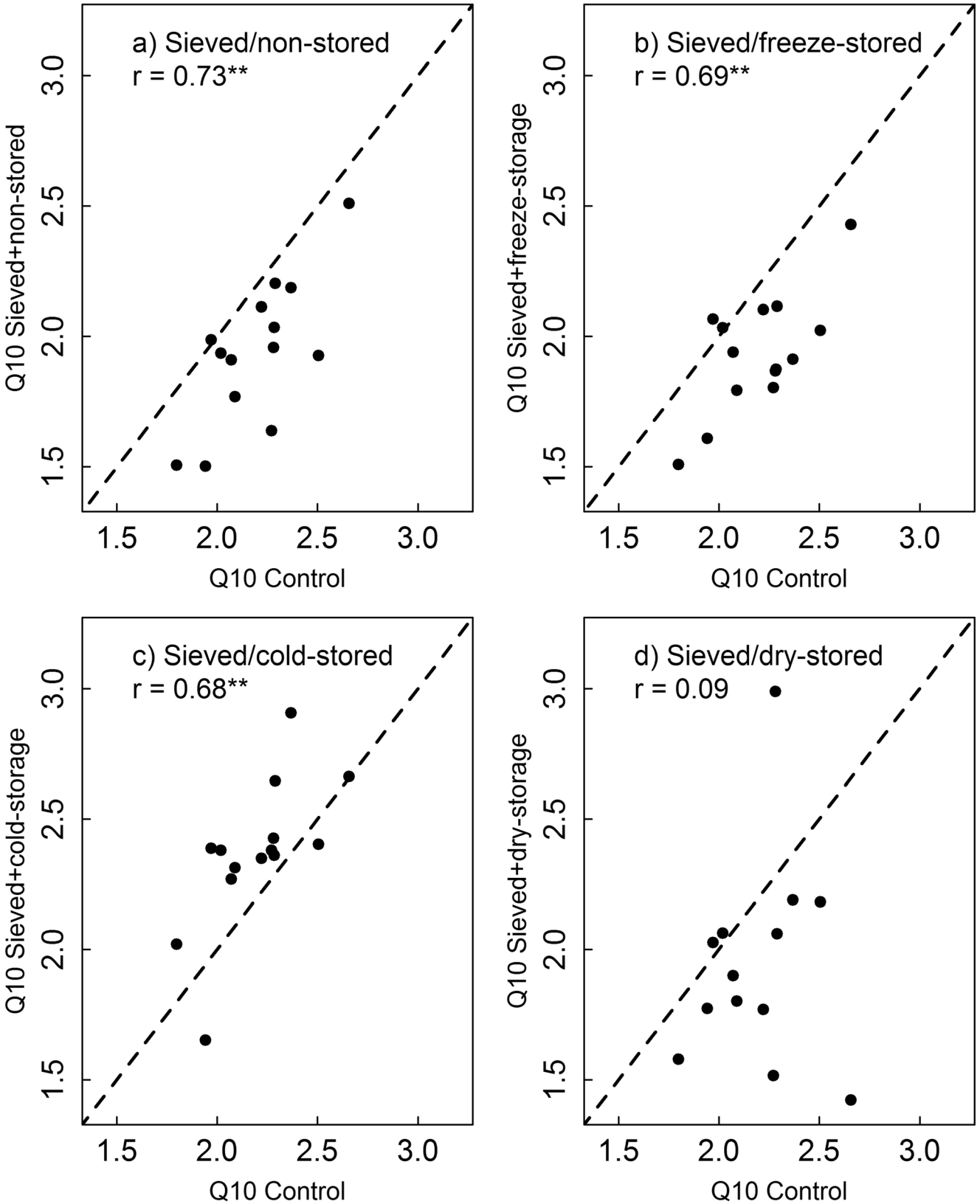

Fig. 2 Relation between Q10 values measured in the four treatments and Q10 values measured in the control, **a** for sieved/non-stored soils, **b** for sieved/freeze-stored soils, **c** for sieved/cold-stored soils, **d** for sieved/dry-stored soils

Yet, as these treatments included sieving, they revealed a tendency towards lower Q10 values compared with the control (Fig. 2, $p = 0.21$ for the effect of freeze-storage and $p = 0.23$ for the effect of dry-storage).

Although Q10 values were slightly over- or underestimated by sieving and/or storage, Q10 values of the sieved/non-stored, sieved/freeze-stored, and sieved/cold-stored treatments correlated with the Q10 values of the control (Fig. 2a–c). Hence, sieving and subsequent freeze- or cold-storage affected all soils similarly and did not change the general rank order from soils with low Q10 values to soils with high Q10 values. This finding did not apply to the sieving/dry-storage treatment, which induced a different rank order of Q10 values compared with the control (Fig. 2d). We conclude that sieving and subsequent cold- or freeze-storage might be acceptable for deriving spatial patterns of Q10 values across the landscape although values might slightly deviate from the undisturbed soil. In contrast, dry-storage should be avoided for the determination of spatial Q10 patterns as it does not affect all samples similarly.

Conclusions

In the case that storage and sieving of soils need to be performed in the run-up to soil respiration measurements, we conclude that sieving field-moist soil with subsequent freeze-storage or short-term cold-storage is most appropriate to conserve original soil respiration rates, their temperature sensitivity (Q10), and their original spatial patterns. A more comprehensive study is required to derive reliable correction factors that could be used for the conversion of lab-derived data into real-world soil respiration rates and Q10 values.

Acknowledgments We thank Peter Grässler, Frederike Zeibig, and Matthias Leenen for their support in field and/or laboratory work.

Funding information Open access funding provided by University of Helsinki including Helsinki University Central Hospital. This work was supported by the Collaborative Research Centre SFB/TR32 "Patterns in Soil-Vegetation-Atmosphere Systems: Monitoring, Modeling, and Data Assimilation" funded by the Deutsche Forschungsgemeinschaft (DFG).

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