# Impacts of soil storage on microbial parameters

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**Abstract.** This review aims to determine the impact of soil storage on microbial parameters (abundance/biomass, activity and various diversity metrics). The literature dealing with the impact of storage practices (cold, freeze, dry, freeze-dry and ambient storage) on soil microbial parameters was analysed in of 76 articles, representing 289 basic data (impact of a given storage practice on a microbial parameter). Globally, more than 75 % of these data showed significant impact of storage on the measured microbial parameters, as compared to those measured on fresh, non-stored soil samples. The storage practices showed various effects on the soil microbial parameters, with sometimes opposite effects across different soil types. For instance, the effects of a given storage practice on different enzyme activities in the same soil were not constant, and moreover, the effects of a given storage practice on a given enzyme activity varied across different soils. Several factors may explain the variability of storage impact (storage duration, soil type/land use, climate condition), but the available data are too scattered to elucidate their respective roles. However, a few storage recommendations can be made, depending on the microbial parameters studied. Storage practices for soil samples, when unavoidable, should be carefully selected according to conditions that prevail in the native soil environment, to microbial parameters that are analysed (even though there is rarely consensus on a best practice), and with different storage practices for different microbial parameters if necessary.

Keywords: preservation, freezing, drying, soil archives, biomolecules

## 1 Introduction

Knowledge about the soil microbial parameters (abundance, biomass, activity, diversity), their spatial distribution and response to various stresses and disturbances is essential for understanding matter and energy fluxes as well as predicting ecosystem services associated to soils (Wagg et al., 2019; Delgado-Baquerizo et al., 2020), although the links between microbial diversity and ecosystem functions remain incompletely elucidated (Nannipieri et al., 2003; Graham et al., 2016). However, analysis of fresh samples can be problematic, especially for soils originating from sites that are located at several hours (or even days) from laboratory facilities, or for soils from sampling sites located at large distances from each other, and that can't be processed rapidly because of transport or shipping constraints (*e.g.* Creamer et al., 2016; Gillespie et al., 2021). Further, archived soils provide an interesting resource for soil scientists to examine long term impacts as those of climate or land use changes ([8], Manter et al., 2017, Hu et al., 2023) or for the inventory of soil properties (Karimi et al., 2018). Soil storage is then inevitable, and the question arises of the best storage option.

The objective of storage is to suppress soil enzyme activities that could alter both biochemical (nutrient or carbon contents) and/or microbial parameters, what inevitably happens if the soil samples are stored at ambient temperature and field

moisture. Suppression of enzyme activity can be achieved by sharply decreasing either water availability (drying of samples), temperature (storage typically at 4°C, -20°C or -80°C), or both (freeze-drying). However, lowering water availability or temperature can have other adverse effects, as both influence the physico-chemical properties of soils (Blake et al., 2000; Sun et al., 2015; Villada et al., 2016; Kühnel et al., 2019), with potential site-specific effects (*e.g.* Kaiser et al., 2015). Drying, by inhibiting solute diffusion, prevents soil microbial activity. But drying also directly impacts on microbial physiology: to face extreme dry conditions, microorganisms can reduce their internal solute potential by accumulating osmolytes or going dormant, and with microorganisms implementing various physiological responses when facing dry conditions (Schimel, 2018). Into more details, bacteria and fungi occupy different water-related niches, with soil fungi being generally more resistant but less resilient than bacteria (*e.g.* Barnard et al., 2013; De Vries et al., 2018). The speed at which the soils are dried as well as the duration of storage (Meisner et al., 2013) could matter. Freezing generates osmotic stress for microbial cells because of increased salt concentration in the liquid phase during ice formation; also, ice crystal formation can damage cells, leading to cell lysis (Mazur, 1984; [54]). Cold storage (generally at 4°C) does not imply, contrary to freezing, an osmotic stress or cell lysis by ice crystal. At low temperatures, proteins are less flexible and cell membrane loses its fluidity, affecting nutrient transport (Chattopadhyay, 2006), and inhibiting replication and transcription (D'Amico et al., 2006); but after an acclimation phase, the synthesis of proteins, and then microbial activity, can restart to some extent (Barria et al., 2013). As for drying, microorganisms implement various physiological responses to cold condition (Barria et al., 2013). Some microorganisms can enter a dormant state or, for cold-adapted organisms, accumulate molecules that help maintaining an active metabolism. Yet, microbial activity (especially the mineralization of easily available organic carbon) may continue even under subzero conditions, especially for soils from cold environments (Jansson and Tas, 2014), leading to reduced C and nutrient availability.

For some analyses, especially those implying incubation, dry samples have to be rewetted, and frozen samples have to be thawed, so that the soil microbial community is re-activated. These steps can induce further effects on soil micro-organisms. Thus, freezing-thawing may result in enhanced N mineralization following the revealing of substrates by ice crystal formation and re-mineralization of lysed microbial cells (*e.g.* [54]). Remoistening of soils that were previously dried (either in the field or in the lab) strongly impacts the microbial community (Bartlett and James, 1980), and causes the germination of fungal spores or the reactivation of bacteria that had resisted to drying under various forms [69]. Also, in dried soils, the rapid increase in soil water potential may cause an osmotic shock, leading to cell lysis or release of intra-cellular osmoregulatory solutes (Fierer and Schimel, 2002). The resulting increase in dissolved organic carbon and nitrogen contents (*e.g.* [31]) may stimulate the growth and activity of microorganisms (Birch et al., 1958). For instance, the recent study by Schroeder et al. (2021) showed that a 14-day pre-incubation (at 45 % water holding capacity and 15°C) had the most pronounced effect on soil microbial respiration rate and microbial biomass, compared to the effects of dry, ambient or freeze storage.

Drying-rewetting or freezing-thawing procedures can also induce a physical disruption of soil aggregates [54], releasing previously protected cells or biomolecules, and further providing a better yield for extraction procedure of biomolecules. Thus, the physico-chemical properties of the soil, including clay content, microaggregate and soil porosity, can explain the occurrence of microsites in which microorganisms can be protected under unfavorable conditions. For instance, [23] suggested that soil clay content, providing potential protective microsites, may enhance the ability to preserve microbial functions under long-term storage or even following drying. Also, freeze-thawing or drying-rewetting procedures could create an expanded niche for some micro-organisms, with both aggregate disruption and microbial cell lysis providing nutrient and carbon to storage-resistant microorganisms ([54]; Fierer and Schimel 2002). Alternatively, [17] suggested that slow-growing organisms (K-strategists) would be favored under disturbance (e.g. freezing-thawing) regime.

The various microbial biomolecules and functional parameters that are used for the characterization of the soil microbial community have different stability, with for instance rRNA degrading very rapidly (Wang et al., 2012), PLFAs being rapidly metabolized following cell death (Hill et al., 2000), and DNA being deemed less recalcitrant than other biomolecules. Consequently, the effects of storage on the soil microbial parameters, resulting from interactions between several parameters such as temperature adaptation, water availability and nutritional status of the soil microorganisms, are complex and difficult to foresee.

There is currently no comprehensive synthesis of the knowledge acquired about the effects of storage practices on the various microbial parameters that are used in soil microbial ecology (although Schroeder et al., 2021 recently proposed a nice synthesis in their introduction). Here I analyzed the studies assessing the effects of soil storage practices on various soil microbial parameters. The usual storage options, including cold (generally at 4°C, COLD), freezing (conservation at generally -20°C, FREEZE), air-drying (DRY), FREEZE-DRYING (which is rarely used because rarely available far from laboratory facilities) or ambient temperature (AMBIENT) storage. Because the corpus of literature dealing with this topic frequently failed to provide adequate data (in particular size effect of storage practices) and gathered very dissimilar results, from several storage practices and microbial parameters, resulting in highly fragmented information, I could not carry a meta-analysis, but rather intended to provide a "state of the art" of the knowledge on this topic. The aim of this study was rather to assess the consistency of the storage impacts (i.e. whether a given storage practice always had the same impact on a given microbial parameter across different soil and climate conditions), and to provide the authors of future studies with contextualized elements.

## 2 Methodology

### 2.1 Data source and collection

A systematic literature review was done. In October 2024, relevant peer-reviewed publications were selected using Web of Science with the following keywords: "soil AND storage AND (microb* OR bacteria* OR fung*) AND (dry OR freez* OR cold OR ambient)" in the field TOPIC. The 1438 articles were screened by relevance and only papers that explicitly assessed the effects of various storage conditions and that compared microbial parameters analyzed on stored soils with those obtained on fresh soils were retained. Additional references were retrieved when citing or being cited by the previous ones. I excluded the articles which assessed uncommon microbial properties, specific microbial groups (rhizobia, pathogens…) as well as papers dealing with substrates other than soils (compost, litter, etc.).

### 2.2 Data screening:

For each paper, I retrieved the following data (in addition to bibliographic information):

- Background information: soil type (forest / arable / mountain / urban…), climate (temperate / tropical / Mediterranean…), storage duration (because authors sometimes assessed the impact of different storage durations, we based our conclusion, when necessary, on the results from the longer storage term.).

- Storage methods: cold storage (+2 to +4°C: COLD); freezing (generally at -20°C, FREEZE); storage after air-drying at ambient temperature (DRY); at ambient temperature and field moisture (AMBIENT); and freeze-drying (FREEZE-DRY). I did not consider freezing at -80 °C additionally to -20°C freezing, because the devices required for deep

freezing are generally lacking at sites far from laboratory facilities. For microbial analyses, especially those based on incubation for activity measurements, that require re-wetting of DRY soils, or thawing of FREEZE ones, the effect of re-wetting or thawing was considered as part of the storage method and the studies generally consider the microbial parameter analyzed on re-wetted (for DRY), thawed (for FREEZE) or warmed soil samples (for COLD). The same was true for methods requiring a pre-incubation period prior or as part of the measurement, to stabilize the *biomass* (fumigation-incubation technique) or to allow the microbial enzymes to reactivate (*e.g.* for substrate induced respiration, SIR): although pre-incubation can impact on soil microbial parameters (*e.g.* [41]), I considered this step as part of the storage procedure. I should have distinguished between the storage procedure and duration, as both may impact soil properties (*e.g.* [46] [60] [72]). However, because of the low number of studies that use various storage duration, and because storage practices often have contrasted effects on different microbial properties, I considered here only the storage practice (with, when relevant, additional comments about the effect of storage duration) with the hope of drawing recommendations for suitable storage.

- Methodological approach: because a given microbial parameter can be estimated using different approaches (for instance, microbial biomass can be estimated either by PLFA extraction, fumigation-extraction, or DNA recovery):

- *CFE*: chloroform-fumigation extraction (for determination of microbial C, N or P);
- *COUNTS*: for direct microbial cell counts (microscopy, cytometry);
- *CULTURE*: Culture-based microbial parameters (cfu counts or morphotypes);
- *DNA*: DNA- (or, rarely, RNA-) based microbial parameters after extraction;
- *INCUBATION*: for parameters estimated following incubation (enzyme activities, CLPP…);
- *PLFA*-based analyses (*PLFA*).

- The microbial parameters that were characterized in the study:

- *abundance*, that can be based on direct *COUNTS*, *CULTURE* (colony-forming units), or *DNA* (qPCR);
- *activity* generally following *INCUBATION* (basal respiration, SIR, denitrification enzyme activity (DEA), community level physiological profile (CLPP), specific soil enzyme activities, …).
- *biomass* that can be based on *DNA* or *PLFA* extraction, or on *CFE* (microbial biomass C, N or P)
- *composition*: list of species / taxa / OTUs detected in the samples, mainly for sequencing (after *DNA* extraction)
- *structure*: species / taxa / OTUs (for sequencing) / group-specific PLFA / amplicon (for molecular fingerprinting) /metabolized substrates (for CLPP)
- *diversity* (OTUs richness or Shannon index; number of cfu morphotypes…)

- Scoring of storage effects: because the impact of several storage methods can be analyzed on several microbial parameters in a paper, I used the impact of each storage method on each microbial parameter as an elemental information (*e.g.* impact of COLD storage on soil basal respiration). For each microbial parameter, the effect of storage practice was scored as follows (when several soils were tested, I scored a single effect than was consistent or not across the tested soils):

- A null score was attributed when the storage did not significantly increase, decrease or change the microbial parameter, compared to that determined in fresh, field-moist, non-stored soil.
- For quantitative parameters (*abundance*, *activity*, *biomass*, *diversity*), a null score was attributed when the storage did not significantly increase or decrease a positive or negative score was attributed when the storage significantly increased or decreased the microbial parameter, respectively, as compared to that of fresh, non-stored soil.
- For qualitative parameters (*structure*, *composition*), the impact of storage practice was recorded as null (when statistically non-significant) or effective when the microbial parameter was significantly different.

- A score variable ("variable*'*") was attributed when the storage had inconsistent effects across different soils, sampling dates or, soil enzyme activities.

When relevant, I distinguished between bacterial, fungal and archaeal parameters (*DNA*- and *CULTURE*-based parameters). When several storage practices were compared, I also noticed their relative effects (and if they were consistent across soil types) as well as whether the ranking between samples was conserved (as compared to the ranking between fresh, non-stored soil samples), when the information was mentioned by the authors. Finally, I based the scoring using author's conclusions.

## 3 Results

### 3.1 Global assessment of storage impacts

A total of 76 articles was used for this synthesis (see References). The number of published articles dealing with this issue has globally increased (10 articles between 1961 and 1980, 16 articles between 1981 and 2000, and 50 articles between 2001 and 2024), with some shifts in the methodological approaches used for the characterization of storage impacts (increasing proportion of studies dealing with *DNA*-based approaches, but still a high proportion of studies using *INCUBATION*-based approaches that were used in more than 50 % of articles) (**Table 1**).

The impact of storage on microbial *abundance* was assessed using *CULTURE* (cfu counts, 8 papers), or *DNA*-based approaches (2 papers, using qPCR). Storage impacts on microbial *biomass* used *CFE* (16 papers), *DNA* yield (4 papers) or *PLFA* (6 papers). Microbial *diversity* and *composition* were investigated using *DNA* (5 and 3 papers for each). The *structure* of the soil microbial community was characterized using *PLFA* (11 papers), or *DNA* (molecular fingerprinting: 8 papers, DNA sequencing: 5 papers), or *CLPP* (6 papers). Finally, the impact of storage on microbial activity, was investigated using various *INCUBATION*-based approaches, with a total of 51 papers (see below).

Many papers investigated the impact of several storage practices, and sometimes on several microbial parameters, so that the synthesis allowed the recovery of a total of 289 data (effect of a given storage practice on a given microbial parameter, in one or several soil samples). COLD (90 data), DRY (82 data), and FREEZE (74 data) were the most frequently studied practices, while AMBIENT and FREEZE-DRY were rarely addressed (34 and 9 data, respectively). Overall, 219 data (75.7 % of the data) showed significant impacts of storage on the studied soil microbial parameter for at least one of the soils tested, while 70 data (24.2 %) showed no significant impact, as compared to microbial parameters measured immediately following sampling on non-stored field moist soil samples. All the practices showed overwhelmingly significant impacts on soil microbial parameters compared to those measured on non-stored soil samples (74 % for COLD, 68 % for FREEZE, 80 % for DRY, 88 % for AMBIENT and 30 % for FREEZE-DRY, but with 9 data available only). Because storage practices are expected to have different impacts on different microbial parameters, these impacts were analyzed by methodological approach and microbial parameter. Significant impacts of storage were recorded for 24 out of 29 data for *CFE*-based parameters (83 %), 15 out of 17 data for *CULTURE*-based parameters (88 %), 38 out of 75 data across all *DNA*-based parameters (51 %), and 113 out of 133 data for *INCUBATION*-based parameters (85 %), and 29 out of 35 data for *PLFA*-based parameters (83 %). Results are shown in **Table 2** and commented below.

### 3.2 *CFE*-based parameters

The effect of storage on microbial *biomass* estimated following chloroform fumigation extraction (*CFE*) was assessed in 16 articles (13 on microbial biomass carbon, MBC, two on microbial biomass phosphorus, MBP, one on microbial biomass nitrogen, MBN, and one on MBC and MBN). The soil microbial *biomass* generally decreased following storage (in 20 out of 29 data), but the conclusions of the studies were very heterogeneous. Eight studies evaluated the impact of COLD storage on these parameters, of which 6 found lower MBC [6] [28] [33] [50] [54], MBN [33] or MBP [60], one study no impact on MBC [45], and one study variable impact according to soil types [44] compared to non-stored soils. Among the six articles addressing the effect of FREEZE storage, [28] [54] [60] concluded to negative effects, [6] [33] to null effect, and [44] to variable effects on microbial *biomass*. Twelve papers assessed the impact of DRY storage on soil microbial *biomass*, with 12 data showing lower MBC, MBP and/or MBN [2] [6] [12] [15] [20] [28] [31] [33] [50] [60], and 2 data showing no effect on MBC after DRY storage [75] [76] compared to non-stored soils. Finally, among the three studies dealing with the impact of storage at AMBIENT temperature, one showed lower MBC [60], one higher MBC [58] and one VARIABLE impact [44], as compared to non-stored soil samples. Among the seven studies exploring the impact of several storage methods on microbial biomass following CFE, the decrease in *biomass* following DRY storage was similar [33] [50] or stronger [6] [28] [60] than following COLD storage, and comparable to [60] or stronger [6] [28] [33] than following FREEZE storage. The conclusions of the studies were highly heterogeneous, with some of them recommending FREEZE (*e.g.* [28] [54]) or AMBIENT storage [60] for the determination of *CFE*-based soil microbial *biomass*, and with DRY storage having the strongest effects compared to COLD or FREEZE storage.

### 3.3 *CULTURE*-based microbial *abundance*

In the eight papers evaluating the impact of storage on culturable microbial counts in soil samples, COLD had negative impact on bacterial *abundance* [28] [49] [55], and null [69], positive [55] or negative [28] [49] impacts on fungal *abundance*. FREEZE had no [37], variable [42] or negative impact [49] on bacterial *abundance*, and positive [37] or negative impact on fungal *abundance* [49]. Negative impact of DRY storage was shown on bacterial counts in [8] [37] [52] and on fungal counts in [37] [52].

### 3.4 *DNA*-based parameters

The impact of soil storage on *DNA*-based parameters was investigated in 18 papers, with several parameters addressed in most of the papers. DNA extract can be used to address soil microbial group-specific *abundance* (using qPCR or qPCR), *biomass* (DNA yield), microbial *diversity*, *composition* or *structure*.

[3] showed no impact of either COLD or FREEZE storage for 10 days on bacterial or archaeal *abundance* using qPCR, while [8] showed a decreased *abundance* of *Pseudomonas* spp. 16S rDNA following long-term DRY storage. The four studies assessing storage effect on soil DNA yield (as proxy of *biomass*) found negative impact of COLD ([28] but not [21]), FREEZE [28] [40] with freeze-thaw], DRY [21] [28], AMBIENT [21], and FREEZE-DRY [67] storage compared to freshly sampled soil. [21] concluded to the absence of one-year COLD storage impact on DNA yield and then to a preference for this storage method. The few studies available for DNA-based *diversity* reported that COLD storage had null ([26], bacteria), negative ([24], Archaea) or variable effects ([19] [24] [25], bacteria and fungi). FREEZE storage showed no [14] [26] or variable impact [25] according to the diversity index considered) on bacterial or fungal [14] diversities, while, DRY storage displayed null [19] or variable [25] effects on bacterial *diversity* but variable effects on fungal *diversity* [19].

AMBIENT storage had null [14] or variable [25] effects on bacterial but variable effect on fungal *diversity* [14], and FREEZE-DRY decreased [67], bacteria] or did not impact [67, AM fungi] molecular *diversity*. [19] found storage impact on both fungal and bacterial molecular *diversity* only when rare taxa were considered. Finally, no study concluded to a better storage method. [25] showed that different storage practices sometimes overestimated and sometimes underestimated bacterial richness, but with minimal impact on Shannon bacterial diversity, with some significant interactions between storage practices, land use type and storage duration.

Three studies only in the synthesis reported the impact of soil storage on bacterial [14] [46] [67], fungal [14] or AM fungal [67] molecular *composition* following *DNA*-sequencing, concluding to no impact of FREEZE [14], or of FREEZE-DRY [67], while FREEZE storage impacted bacterial *composition* in [46] and AMBIENT storage both fungal ([14] with variable effects) and bacterial [14] [46] *composition*. [14] and [46] recommended FREEZE rather than AMBIENT storage. [25] also evidenced substantially significant effects of all storage conditions on the composition of the soil bacterial communities, with the strongest compositional shift following DRY and AMBIENT storage in some soils.

Molecular fingerprinting was used for the characterization of community *structure* in eight articles. No impact of COLD storage was reported on community *structure* for Bacteria [3] [32] [57] or Archaea [3]. FREEZE storage had generally no impact on bacterial fingerprinting [3] [32] [57] [65], while significant impacts were reported for archaeal ([40] but not [3]) and fungal [9] molecular fingerprints. DRY storage had generally significant impact on bacterial ([8] [57] [61] [65], but not [32]), or fungal [9] *structure*. Finally, [57] reported no impact of AMBIENT storage on bacterial T-RFLP patterns. Overall, more frequent and stronger impacts were reported following DRY storage compared to COLD and FREEZE, especially for bacteria, while archaea and fungi could be more sensitive to freeze storage. For the characterization of community structure based on sequence data: among the five available studies, [24][26] [66] (for bacteria), [19] (fungi) and [24] (Archaea) found no significant impact of COLD, FREEZE or DRY storage impacts on fungal or bacterial community *structure* except for a significant COLD storage effect on bacterial community *structure* in different soil types [19] and [24] evidences shifts in the bacterial and fungal community structure, respectively, following COLD storage. [25] found that all storage practices impacted the structure of the soil bacterial community, depending on soil types, and with strongest compositional shift or DRY and AMBIENT storage

### 3.5 *INCUBATION*-based microbial parameters:

Six studies evaluated the impact of storage on the outcomes of CLPP (Biolog or MicroResp™ analyses). [9] found that both DRY and FREEZE storage impacted the soil microbial metabolic *activity* but with a stronger impact of DRY over FREEZE storage. [64] found no effect of COLD storage on *activity* using MicroResp™, compared to that of fresh soil samples. All studies characterizing the soil microbial functional *structure* using CLPP showed significant impact of the various storage practices [9] [17] [18] [23] [49] [66], but with a stronger impact of COLD [17] [49] and AMBIENT storage [17] compared to that of FREEZE storage. [66] also reported various impacts of COLD and DRY storage across different soil types, while FREEZE had more consistent effects in this study.

The impact of storage on various soil microbial (basal and substrate-induced) respiration was evaluated in 18 and 8 papers, respectively. COLD storage resulted in either similar [34] [45], enhanced [5] [27] [32] [66] or reduced [6] [54] basal respiration rates. COLD storage sometimes showed comparable effects on SIR with enhanced [5] [32] or reduced [6] [51] values, but with decreased SIR in [45] (compared to a null effect on basal respiration) and unchanged SIR after 13 months of storage in [54] (compared to a negative effect on basal respiration measured in the same soils). FREEZE storage generally enhanced basal respiration [6] [22] [27] [32] [66] except in [34] (no impact) and [54] (reduced respiration rates) compared to that of fresh soils. FREEZE storage showed null [40] [54], variable [6] or enhancing effects ([32] similar to basal

respiration) on SIR rates. Thirteen studies investigating the effect of DRY storage on soil microbial basal respiration concluded to either null [23] [66] [75] [76], positive [12] [32] [34] [50] [68], negative [27] [37] or variable effects ([6] [70]), while the three studies characterizing SIR showed either enhanced ([32], like for basal respiration) or variable SIR rates ([6] [70], similar to basal respiration]) following DRY storage, compared to those in non-stored soils. Finally, the only study addressing the impact of AMBIENT storage showed decreased [5] rates in both basal respiration and SIR. Studies that compared several storage methods concluded to various conclusions, with recommendations that often diverge. It is worth noting that storage practices impacted differently basal respiration and SIR measured on same soil types ([6] [32] [50] [54]). Seven studies assessed the impact of soil storage on potential denitrification *activity* (DEA). COLD storage had either null [4], positive [7] or negative [51] [54] effects on DEA rates. FREEZE storage resulted in enhanced [4] or decreased [54] DEA rates, while DRY storage consistently enhanced [30] [38] [70] DEA rates, and AMBIENT storage decreased [4] [30] DEA rates. When comparing COLD and FREEZE, [10] [54] concluded to stronger impact of COLD over FREEZE on DEA, while [4] concluded to lower impact of COLD over FREEZE. These authors emphasized that responses to storage practices were dependent on land use and time.

Various other soil activities were assessed by few studies [12] [16] [27] [31] [40] [43] [44] [59] [62] [69] and often concluded to variable impacts of a given storage practice on activities (*e.g.* [27] [44] [50] [69]).

Finally, twenty-one studies addressed the impact of soil storage on various soil enzyme *activity* in one or several soils, gathering a total of 43 data about the effect of a storage practice on one or several enzyme activities (9 data for COLD, 12 for FREEZE, 15 for DRY, 6 for AMBIENT, and 1 for FREEZE-DRY). When considering the author's conclusion across all enzyme activities: COLD mainly decreased enzyme activities [1] [5] [10] [28] [56] [60], although [13] [25] and [36] found variable effects across different soils and/or enzymes. FREEZE storage resulted in either decreased [1] [10] [28] [43] [60], less frequently enhanced [22] [39], or similar enzyme activities [56] [73] and with variable effects in [13] [25] and [65] on various enzyme activities and/or soil types. DRY storage showed negative [1] [10] [28] [60] [72], null [73] [74] [76], positive [23] [39] or variable [11] [25] [35] [53] [65] effects. Preservation at AMBIENT temperature decreased enzyme activities [5] [43] [56] [60] except for null impact in [73]. Finally, soil enzyme activity decreased following FREEZE-DRY storage in [72]. Comparison between storage practices yielded contrasted results. FREEZE was sometimes identified as more suitable for measurement of soil microbial enzyme activities than DRY [1] [28] [39] [65] or than COLD [1] [56], but other studies identified COLD as the preferred practice [10] [28]. [60] recommended AMBIENT to other practices for long-term (more than two 2 weeks) storage.

In a given study, storage practices sometimes yielded contrasted impacts on different soil enzyme activities. For instance, in [13], COLD and FREEZE storage showed no impact on β-glucosidase and peroxidase activities, but variable impacts on N-acetyl-glucosaminidase, phenoloxidase and phosphatase (see also [23], [28] [35] [65]). Storage practices also had inconstant impacts on soil enzyme activities across different soil types (e.g. [25], [36]). Because studies often investigate several enzyme activities (one to ten, median 3.0 per study) that can respond differently, in a second step I analyzed the impacts of storage practices on specific enzyme activities. I report here the conclusions for the main soil enzyme activities (for which at least five studies are available) in **Table 3.** Storage practices generally significantly impacted all soil enzyme activities, as compared to those measures in fresh, unstored samples. Regarding the six main enzyme activities, twenty-one papers gathering a total of 105 individual data, *i.e.* impact result of a storage practice on a given soil enzyme activity in one or several soils) were analyzed. DRY was the most frequently practice addressed (39 data) and AMBIENT the less frequently addressed, with 9 data only. Across all practices and enzymes, the impact of storage resulted in variable effects across soil types (53 data), reduced (35 data), or enhanced (a single study, [53]) enzyme activities as compared to those measured in

fresh, non-stored soil samples. Enzyme activity was unaffected for 16 data out of 105, i.e. in 15 % of analyzed data (**Table 3**).

For dehydrogenase *activity*, (5 articles) gathered no [1] or variable effects [5] [10] of COLD storage, negative [43] or variable effects [10] [22] of FREEZE storage, negative [1] or variable effects [10] of DRY storage, and negative [43] or variable effects [5] of AMBIENT storage. For arylsulfatase *activity* (6 articles): COLD storage resulted in negative [56] or variable effects [28] [36], FREEZE storage in variable [28] [65], or null effects [56], and DRY storage in negative [11] [28], variable [65] or null effects [35]. [56] recommended FREEZE over COLD storage for this enzyme. The six articles using glucosaminidase *activity* found variable [13] [25] [60], negative [36] or null effect [28] of COLD, variable [13] [25] [28] [60] or negative effect [39] of FREEZE, and negative [23] [25] [39] [60] or variable effect [28] of DRY storage, and negative effect of AMBIENT storage [25] [60], with no best storage practice identified. Glucosidase activity was assessed in twelve studies, showing variable [25] [28] [28][60] or null [1] [13] effects or of COLD, variable [1] [25] [28] [60] [65] or null effect [13] of FREEZE, negative [1] [11] [25] [60] [65] [72], variable [28] [76] or null effect [74] of DRY storage, and variable effect of AMBIENT storage. The impact of storage practices was assessed on several types of phosphatases across 16 studies (see footnote **Table 3**). The impact COLD storage was recorded as negative [5] [36] [60, phosphomonoesterase], variable [13] [25] [28, alkaline phosphatase] [60, phosphor-mono- and di-esterases], or null [1] [28, acid phosphatase]. The impact of FREEZE was negative [28, alkaline phosphatase] [39] [65, phospho-monoesterase] or variable [1] [13] [25] [28, acid phosphatase] [60, phosphor-mono- and diesterase] [65]. Finally, AMBIENT storage decreased phosphatase activity or had variable effects in [54] [60]. COLD was the most conservative practice in [1] and [28] over FREEZE and DRY. But overall, a given practice could have different impacts on different phosphatase activities measured in a same set of soil samples (acid *vs* alkaline phosphatase [28], phosphomonoesterase *vs* phosphodiesterase [60] [65]). Finally, among the seven studies evaluating soil storage impact on urease *activity*: Null [23] or variable effect [28] of COLD storage were reported, null [73], negative [1] or variable effect [28] of FREEZE, and variable [23] [28] [76], null [73] [74], negative [1] or positive effect [53] of DRY storage. As for phosphatase, COLD was the most conservative practice for urease activity in [1].

### 3.6 *PLFA*-based parameters

Eleven studies evaluated the impact of soil storage on *PLFA*-based microbial parameters. Storage had various impacts on soil microbial *PLFA-biomass*. COLD storage showed negative [28] [36] [66], null [41] [47] or positive [59] impacts. FREEZE storage had either non-significant [47] or negative impacts [28] [66], and DRY storage had either no [66] or negative effect [28] on soil *biomass*. Finally, storage at AMBIENT temperature showed no [66], negative [41] or positive [59] impacts, and the only study reporting the impact of FREEZE-DRYING concluded to a negative impact on soil *biomass* as compared to non-stored soils [71]. The conclusions of the three studies comparing several storage methods were not consistent, with [47] showing no impact of COLD or FREEZE, [59] showing similar enhancement of *PLFA-biomass* following COLD and AMBIENT storage, [66] showing stronger impact of COLD compared to FREEZE and DRY storage, and finally [28] showing stronger decrease following DRY compared to COLD or FREEZE storage. Regarding the *structure* of the microbial community based on the relative abundance of *PLFA* group-specific biomarkers: the six studies about COLD storage reported significant [29] [47] [48] [59], variable [28 on FAME] or null [41] effects. The impact of FREEZE storage was reported as significant in three studies [29] [47] [48] and variable in one study [28 on FAME]. The five studies using DRY storage reported significant impacts on the soil microbial community *structure* [20] [28] [29] [48] [63]. AMBIENT and FREEZE-DRYING storage impacted the *PLFA*-based *structure* of the soil community in all reported studies ([41] [59] [63] and [29] [71], respectively). Among the studies reporting several storage practices, [29] and [47] concluded

to lower impact of FREEZE compared to COLD or DRY storage [29] while [48] concluded to comparable impact of these three storage methods on PLFA patterns.

## 4 Discussion

Overall, and considering to the wide range of microbial parameters used in soil ecology and methodological approaches available to characterize these parameters, the literature addressing the impacts of soil storage on soil microbial parameters is rather sparse, even though storage is a common, even widespread practice. The present review suggest that these impacts are widespread and frequent (almost 76 % of published data), across all microbial parameters and storage practices. It would have been interesting to evaluate and compare the effect sizes of storage impacts, but the required data were not always available, particularly for older articles. Several studies suggest that these impacts can be strong. For instance, [31] found that soil MBC and MBN decreased by two-three times in dried mountain-meadow soils, compared to those measured in fresh soils. [8] reported that "archived [dried] soils […] contained dramatically less pseudomonad DNA than fresh soil". [17] concluded that "substantial changes can occur to the soil microbial community functions, regardless of the kind of storage […depending on] the profile and sampling depth", and "a great sensitivity of CLPPs to storage treatment". Except for FREEZE-DRY storage with 9 data only, FREEZE recorded the lowest impact frequency (with 68 % of significant effects) while AMBIENT and DRY storage more frequently impacted the microbial parameters (86 and 80 % of data, respectively). This result shall be treated with caution, as these different practices are used preferentially for certain parameters (*e.g.* FREEZE for DNA-based parameters). Therefore, data on the impact of all practices are not available in an equivalent way for all parameters. Also, some authors have published several studies on the impact of a practice on certain microbial parameters for a given soil type (*e.g.* [74] [75] [76] on Mediterranean soils), thus distorting the representativeness of the available data.

This review identified three main factors that explain the variability of impacts of the different storage practices: duration of storage, soil type/land use, and climate conditions.

Although this factor has not been evaluated as such, the duration of storage generally influenced its impacts. For instance, [10] showed that upon the long term (> 12 weeks), the differences in enzyme activities between soil samples stored under different conditions became less pronounced. [14] and [46] even showed that DNA thaw time and storage duration can impact soil microbial molecular parameters, respectively. Also, several authors recommend incubation/conditioning of the soil samples following storage before microbial analyses [32] [54], although new microbial groups (*i.e.* groups that were not detected in fresh samples) can appear following incubation, *e.g.* in [32]. Incubation (conditioning) under moist condition did [66] or did not ([43], West et al., 1986) allow the restoration of the soil microbial parameters. For instance, [19] showed that the soil microbial respiration and C biomass retrieved values similar to those of fresh soils after a few days of pre-incubation under moist condition, even for 36-years old soil samples [23]. However, this issue of preincubation effects is largely underestimated and would require more consistent studies.

This review also illustrates the wide differences in storage impacts across different soil types and/or land use. Storage impacts on various soil microbial parameters varied according the soil types (*e.g.* [6] [10] [32] [70]. The recent study by Lane et al. [25] showed that various parameters of the soil bacterial community were significantly affected by the interactions between storage, land use, and sometimes storage duration. Regarding the role of soil type: some authors proposed a presumable effect of soil textural parameters [28] [65], with high clay content providing abundant microsite to soil microorganisms that may improve the preservation of microbial parameters following storage-associated disturbance [12] [23]. It was also suggested that soils with high amount of organic matter could better resist to storage impact (e.g. [12]).

Gonzales-Quinones et al. [18] hypothesized that the microbial parameters in soils with low organic C contents would be more affected by storage, but they observed the opposite, concluding that microorganisms associated to large recalcitrant organic C pools (grassland, woodland) would be more subjected to death during storage than microorganisms relying on more easily degradable C sources [18]. Indeed, younger and more active microorganisms may be more sensitive to drying-rewetting or freezing stress than more stable microbial biomass. In line, Gram positive bacteria (especially actinomycetes, firmicutes) have been considered to be more stress-tolerant (*e.g.* [32]) than Gram negative bacteria.

The third factor identified to explain the variability of storage impacts in different studies was climate conditions. Soils frequently exposed to DRW may be more adapted to drought stress because of the selection of microbial groups that are more resistant to osmotic stress (Fierer and Schimel, 2002; Evans and Wallenstein 2003) and then less impacted by air-dry storage (*e.g.* [20]). For similar reasons, soil samples yielded during summer season might be less affected by dry storage than those yielded during the cold, humid fall and winter season, as shown by [75] on Mediterranean forest soils. The sampling season could thus influence storage impacts [1]. Indeed, Evans and Wallenstein (2014) proposed that precipitation/soil moisture regime alters ecological strategies of the soil microbial community both through changes in community composition and strategy shifts within taxa. They found that a decade of more frequent exposure to intensified rainfall patterns increased the proportion of taxa exhibiting a stress tolerant strategy. On the contrary, flooded (*e.g.* paddy) soils would be more impacted following air-drying preservation (*e.g.* [66]). Similarly, soil microbial communities in soils that regularly undergo *in situ* freezing might be less impacted by storage at -20°C, presumably due to the accommodation of the microbial community to regular annual freezing [46] [54], and inversely, microbial communities that are not naturally exposed to cold temperature (*e.g.* tropical soils) would be more sensitive to FREEZE storage [60]. In their recent article, [25] suggested defining regionally standardized practices for soil storage, and some authors (*e.g.* [25]) some authors note in their conclusion that the results of their study would be valid for studies carried out in similar climates.

The effects of storage might be tolerable if the storage procedure has the same proportional effect on soil samples yielded across various sites or submitted to different experimental treatments (*i.e.* if the ranking / similarity between sample's microbial parameters is conserved following storage). Indeed, some authors explicitly mention in their conclusion that differences (ranking) between microbial parameters from different soil types or ecosystems are preserved independently of the storage method (*e.g.* [10] [18] [34] [35] [61]) or of the storage duration ([18] [65]). However, several studies suggest that the storage practices do not impact various soil microbial parameters in a similar way (*e.g.* [6] [70] for SIR, [11] [13] for soil enzymes, [28] for PLFAs, [66] for MicroResp™ CLPP).

As anticipated, the impacts of storage practices differed between microbial parameters, with frequent inconsistent effects reported across different studies. In the light of the results, it may be asked whether it is possible to provide recommendations for the storage of samples in order to assess the various microbial parameters. The fragmentation of available information and the variability of storage effects observed make this a tricky exercise.

Surprisingly, studies based on DNA sequence analysis and reporting the effects of soil storage on microbial community structure or composition are very rare, while this practice is very common. Generally, different storage times and freezing temperatures did not drastically change community structure and composition [26] [46]. FREEZE appear to the best storage practices, but here again, it is difficult to draw conclusions from the small number of studies available. Statistics are also impossible due to the small number of articles available, but a few data suggest that bacteria and fungi are equally impacted, although they sometime respond differently [14] [19] [67]. Studies dealing with Archaea [3] [40] are too sparse to draw conclusions about this group. Some studies showed that FREEZE storage can even have some effects on molecular microbial parameters [25] [28] [40] [48] contrary to what generally accepted [28], and although these impacts were detected

at higher taxonomic levels [14] [46] or when rare taxa were considered [19]. This last point suggests that, technological advances, allowing for more resolved taxonomic characterization, could also reveal hitherto unsuspected effects of storage practices on microbial communities. The few available data showed that storage generally impacted some microbial clades that become extinct or below the detection limit after a few days of storage only and in an unpredictable way [46]. For instance, Finn et al. (2023) showed that, for Bacteria, the relative abundance of Acidobacteria, Actinobacteria and Thermoproteota was more affected by storage practice than Bacteriodota, Firmicutes and Planctomycetota. In line, the recent study by Hu et al. (2023) suggests that FREEZE long-term storage of soil samples destabilize bacterial co-occurrence patterns. These authors proposed that the removal of relic DNA (extra-cellular and dead microbe DNA) with chemical treatment would improve the accuracy of bacterial diversity in long-term frozen soil samples. Finally, certain commercial preservatives could be useful in limiting storage impact on DNA (Smenderovac et al., 2024).

Regarding PLFAs, biomass measured using PLFAs was generally underestimated following all storage practices. The data suggest that FREEZE or COLD should be preferred over DRY storage. The effect of storage on PLFAs could be explained by mechanism of temperature adaptation or response to stress, including a decrease in the degree of unsaturation ([41], see also Kaneda, 1991). Using PLFA biomarkers, [20] found DRY storage to favor Gram-positive (over Gram-negative) bacteria, and to increase the bacteria:fungi ratio, but [29] concluded that DRY storage of flooded soils increased the Gram-negative bacteria.

Microbial parameters determined following INCUBATION of the soil samples were frequently impacted by storage (with about 86 % of 133 data indicating a significant impact). Impacts of storage practices on basal respiration and potential microbial activities (SIR, DEA) we often inconsistent across different soil types, with no consensus for a best storage option. Also, and worryingly, BR and SIR, when assessed in the same study, could present opposite responses to storage practices, suggesting that soil samples should be stored in different ways for these analyses (see below). Similarly, for CLPP analysis, the available literature report inconsistent storage effects. For microbial activity measurements, the storage condition may affect activity rates but also other kinetic parameters. For instance, [5] showed that the latent time observed during the first hours of respiration analysis increased with storage time for soils stored at 4 or 37°C. Finally, regarding soil enzyme activities, some authors of recommend COLD or FREEZE storage as the most conservative, and DRY storage as the less desirable practice [1] [28] [56] [65] although DRY storage could be suitable in some cases [11] [74]. However, the present synthesis concludes to a global strong and unpredictable impact of storage practices on soil enzyme activities, with highly variable effects across enzyme activities and soil types. In almost half of the data on individual enzyme activities (Table 3, 41 data out of 83), storage of the soil samples had variable impacts on a given soil enzyme activity across different soils. This suggest that any storage should be strongly avoided in studies dealing with enzyme activities in different soil types and origins.

**Conclusions**

In a large majority of studies, the various soil microbial parameters were significantly impacted by storage, and these impacts often varied across different storage practices, microbial parameters and soil types. Of course, storage cannot always be avoided, and it would be unrealistic to recommend avoiding it. and especially when different soil types or soils of various geographical origins are compared. Although some studies suggest that preservation at field moisture and room temperature might be the best option for a short-term storage, this should operate for a few days only. If the soil samples cannot be processed rapidly, the storage options have to be carefully considered. Because the different microbial parameters do not respond similarly to the various storage options, multiple sample storage methods may be used. This review also highlights the need to couple the storage option with the abiotic conditions (mean annual temperature, precipitation regime…) that prevail in the native soil environment (see also Sheppard and Addison (2007) suggesting that storage

practices cannot be universal). Rhymes et al. (2020) recently proposed a procedure to determine the best storage method for soils C and N determination; they recommend maximum storage length and suggest, as other authors (e.g. [24]) running a pilot study (*e.g.* Lee et al. 2021) to determine best storage practice for a given soil type and microbial parameter, and including the results in the publications: one can only fully support this later recommendation. If such a pilot study is not feasible, the authors should systematically mention possible storage-related biases.

The present analysis clearly shows that, based on data available in the literature, it is very risky to prescribe a maximum storage duration for the determination of microbial parameters for all soil types, given the heterogeneity of author's conclusions and recommendations. The good news is that the storage effects generally do not always impair our capacity to assess treatment effects on soil microbial parameters, at least for a given soil type submitted to different treatments (plant composition, management practice…). The challenge of soil storage is more critical for studies dealing with multiple locations / soil types, as the effect of storage on microbial properties vary with soil types.

## Author contribution

NF conceived the study, analyzed the bibliography and wrote the article.


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**Table 1- Evolution of the number of articles and of methodological approaches used for the characterization of storage impacts on soil microbial parameters (1961-2024)**. The number of articles (*n*) listed in this table is 104 (rather than 76) because some articles use several methodological approaches.

| Period (number of articles) | *CFE* | *CULTURE* | *DNA* | *INCUBATION* | *PLFA* |
|---|---|---|---|---|---|
| 1961-1970 (*n* = 3) | | [55] | | [22] [56] | |
| 1971-1980 (*n* = 7) | [44] | [37] [52] [69] | | [37] [38] [44] [69] [72] [73] | |
| 1981-1990 (*n* = 3) | | [58] | | [4] [53] | |
| 1991-2000 (*n* = 13) | [45] [50] [54] | [28] [49] | [21] | [5] [28] [30] [45] [49] [50] [51] [54] [62] [68] | [41] [48] |
| 2001-2010 (*n* = 22) | [6] [12] [15] [20] [27] [60] [75] [76] | [8] [40] | [8] [26] [27] [40] [61] [65] | [6] [10] [12] [13] [17] [18] [27] [40] [43] [59] [60] [65] [74] [75] [76] | [20] [27] [29] [59] [71] |
| 2011-2024 (*n* = 28) | [2] [31] [33] | [42] | [3] [9] [14] [19] [24] [25] [32] [46] [57] [66] [67] | [1] [7] [9] [11] [16] [23] [25] [31] [32] [34] [35] [36] [39] [64] [66] [70] | [36] [47] [63] |

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**Table 2- Synthesis of the impacts of storage practices (COLD, FREEZE, DRY, AMBIENT and FREEZE-DRY) on the soil microbial parameters as compared to parameters estimated on fresh, non-stored soils.** The changes are scored as no impact Ø (no significant change), increase ⇉ or decrease ⇇ (for quantitative parameters, *i.e.* higher and lower parameter values, respectively), change ≠ (for significant change on non-quantitative parameters), or variable ⤮ (when storage had inconsistent changes across soil samples or soil enzyme activities). References for studies that have shown significant impacts of storage are in bold. For DNA-based analyses, the distinction was made between analyses targeting bacteria (B), Archaea (A) and fungi (F).

| **Methodological approach** | **Microbial Parameter** | **COLD** | **FREEZE** | **DRY** | **AMBIENT** | **FREEZE - DRY** |
|---|---|---|---|---|---|---|
| CFE | *biomass* (C, N, P) | Ø [45]<br>⇉ **[6] [28] [33] [50] [54] [60]**<br>⤮ **[44]** | Ø [6] [33]<br>⇉ **[28] [54] [60]**<br>⤮ **[44]** | Ø [75] [76]<br>⇉ **[2] [6] [12] [15] [20] [28] [31] [33] [50] [60]** | ⇉ **[60]**<br>⇉ **[58]**<br>⤮ **[44]** | |
| CULTURE | *abundance* (bacteria) | ⇉ **[27] [49] [55]** | Ø [37]<br>⤮ **[42]**<br>⇉ **[49]** | ⇉ **[8] [37] [52]** | | |
| CULTURE | *abundance* (fungi) | Ø [69]<br>⇉ **[55]**<br>⇉ **[27] [49]** | ⇉ **[37]**<br>⇉ **[49]** | ⇉ **[37]-[52]** | | |
| DNA | *biomass* | Ø [21]<br>⇉ **[28]** | ⇉ **[28] [40]** | ⇉ **[21] [28]** | ⇉ **[21]** | ⇉ **[67]** |
| DNA | *abundance* (bacteria) | Ø [3] | Ø [3] | ⇉ **[8]** | | |
| DNA | *abundance* (archaea) | Ø [3] | Ø [3] | | | |
| DNA sequencing | *diversity* (bacteria) | Ø [26]<br>⇉ **[24]**<br>⤮ **[19] [25]** | Ø [14] [26]<br>⤮ **[25]** | Ø [19]<br>⤮ **[25]** | Ø [14]<br>⤮ **[25]** | ⇉ **[67]** |
| DNA sequencing | *diversity* (fungi) | ⇉ **[24]**<br>⤮ **[19]** | Ø [14] | ⤮ **[19]** | ⤮ **[14]** | Ø [67] AMF |
| DNA sequencing | *diversity* (archaea) | ⇉ **[24]** | | | | |
| DNA sequencing | *composition* (bacteria) | | Ø [14]<br>≠ **[46]** | | ≠ **[14] [46]** | Ø [67] |
| DNA sequencing | *composition* (fungi) | | Ø [14] | | ⤮ **[14]** | Ø [67] AMF |
| DNA fingerprinting | *structure* (bacteria) | Ø [3] [32] [57] | Ø [3] [32] [57] [65] | Ø [32]<br>≠ **[8] [57] [61] [65]** | Ø [57] | |
| DNA fingerprinting | *structure* (fungi) | | ≠ **[9]** | ≠ **[9]** | | |
| DNA | *structure* (archaea) | Ø [3] | Ø [3]<br>≠ **[40]** | | | |
| DNA sequencing | *structure* (bacteria) | Ø [24][26] [66]<br>≠ **[19] [25]** | Ø [26] [66]<br>≠ **[25]** | Ø [19] [66]<br>≠ **[19] [25]** | ≠ **[25]** | |
| DNA sequencing | *structure* (fungi) | Ø [19]<br>≠ **[24]** | | Ø [19] | | |
| DNA sequencing | *structure* (archaea) | Ø [24] | | | | |

**Table 2 (continued)**

| Methodological approach | Microbial Parameter | COLD | FREEZE | DRY | AMBIENT | FREEZE - DRY |
|---|---|---|---|---|---|---|
| INCUBATION | *activity* (CLPP) | Ø [64] | ⇉ **[9]** | ⇉ **[9]** | | |
| INCUBATION | *structure* (CLPP) | ≠ **[17][18][49]** ⤮ **[66]** | ≠ **[9] [17] [49] [66]** | ≠ **[9] [23]** ⤮ **[66]** | ≠ **[17]** | |
| INCUBATION | *activity* (basal respiration) | Ø [34] [45] ⇉ **[5] [27] [32] [66]** ⇉ **[6] [51] [54]** | Ø [34] ⇉ **[6] [22] [27] [32] [66]** ⇉ **[54]** | Ø [23] [66] [75] [76] ⇉ **[12] [32] [34] [50] [68]** ⇉ **[27] [37]** ⤮ **[6] [70]** | ⇉ **[5]** | |
| INCUBATION | *activity* (SIR) | Ø [54] ⇉ **[5] [32]** ⇉ **[6] [45] [51]** | Ø [40] [54] ⇉ **[32]** ⤮ **[6]** | ⇉ **[32]** ⇉ **[50]** ⤮ **[6] [70]** | ⇉ **[50]** ⇉ **[5]** | |
| INCUBATION | *activity* (DEA) | Ø [4] ⇉ **[7]** ⇉ **[51] [54]** | ⇉ **[4]** ⇉ **[54]** | ⇉**[30][38] [70]** | ⇉ **[4] [30]** | |
| INCUBATION | *activity* (various) | ⇉ **[16] [59][62]** ⤮ **[27] [44] [50] [69]** | Ø [40] ⇉ **[31]** ⇉ **[43]** ⤮ **[44] [50]** | Ø [16] ⇉ **[12]** | ⇉ **[43] [59]** ⇉ **[50]** ⤮ **[44]** | |
| INCUBATION | *activity* (enzyme activities) | ⇉ **[1] [5] [10] [28] [56] [60]** ⤮ **[13] [25] [36]** | Ø [56] [73] ⇉ **[22] [39]** ⇉ **[1] [10] [28] [43] [60]** ⤮ **[13] [65] [25]** | Ø [73][74][76] ⇉ **[23] [39]** ⇉ **[1] [10] [28] [60] [72]** ⤮ **[11] [25] [35] [53] [65]** | Ø [73] ⇉ **[5] [43] [56] [60]** ⤮ **[25]** | ⇉ **[72]** |
| PLFA | *biomass* | Ø [41] [47] ⇉ **[59]** ⇉ **[28] [36] [66]** | Ø [47] ⇉ **[28] [66]** | Ø [66] ⇉ **[28]** | Ø [66] ⇉ **[59]** ⇉ **[41]** | ⇉ **[71]** |
| PLFA | *structure* | Ø [41] ≠ **[29] [47] [48] [59]** ⤮ **[28]** | ≠ **[29] [47] [48]** ⤮ **[28]** | ≠ **[20] [28] [29] [48] [63]** | ≠ **[41] [59] [63]** | ≠ **[29] [71]** |

**Table 3- Synthesis of the impacts of storage practices (COLD, FREEZE, DRY, AMBIENT) on the main soil enzyme activities** (as compared to those estimated on fresh, non-stored soils). The changes are scored as no impact Ø (no significant change), increase ⇉, decrease ⇇, or variable ⤮ (when storage had inconsistent changes across soil samples). References for studies that have shown significant impacts of storage are in bold.

| **Soil enzyme activity** | **COLD** | **FREEZE** | **DRY** | **AMBIENT** |
|---|---|---|---|---|
| Dehydrogenase | Ø [1]<br>⤮ **[5] [10]** | ⇉ **[43]**<br>⤮ **[10] [22]** | ⇉ **[1]**<br>⤮ **[10]** | ⇉ **[43]**<br>⤮ **[5]** |
| Arylsulfatase | ⇉ **[56]**<br>⤮ **[28] [36]** | Ø [56]<br>⤮ **[28] [65]** | Ø [35]<br>⇉ **[11] [28]**<br>⤮ **[65]** | ⇉ **[56]** |
| Glucosaminidase | Ø [28]<br>⇉ **[36]**<br>⤮ **[13] [25] [60]** | ⇉ **[39]**<br>⤮ **[13] [25] [28] [60]** | ⇉ **[23] [25] [39] [60]**<br>⤮ **[28]** | ⤮ **[25] [60]** |
| Glucosidase | Ø [1] [13]<br>⤮ **[28] [36] [60] [25]** | Ø [13]<br>⤮ **[1] [25] [28] [60] [65]** | Ø [74]<br>⇉ **[1] [11] [25] [60] [65] [72]**<br>⤮ **[28] [76]** | ⤮ **[25]** |
| Phosphatase | Ø [1] [28][1]<br>⇉ **[5] [36] [60][1]**<br>⤮ **[13] [25] [28][2] [60][2]** | ⇉ **[39] [28][2] [65][1]**<br>⤮ **[1] [13] [25] [28][1] [60][1,2] [65][2]** | Ø [74] [76]<br>⇉ **[1] [11] [28][2] [39] [60][1,2] [65][1,2]**<br>⤮ **[25] [28][1] [35] [53]** | ⇉ **[5] [60][1]**<br>⤮ **[60][2]** |
| Urease | Ø [1]<br>⤮ **[28]** | Ø [73]<br>⇉ **[1]**<br>⤮ **[28]** | Ø [73] [74]<br>⇉ **[1]**<br>⇉ **[53]**<br>⤮ **[23] [28] [76]** | |

[1] and [28][2] refer to alkaline phosphatase.

[5] refers to phosphoesterase.

[11] [13] [25] [28][1] [35] [74] and [76] refer to acid phosphatase.

[36] refers to both acid and alkaline phosphatases

[53] refers to phosphatase without further clarification.

[60][1] and [65][1] refers to phosphomonoesterase.

[60][2] and [65][2] refers to phosphodiesterase.