Reviewer #1 (Remarks to the Author):

Hi there

I enjoyed reading your manuscript. It is well written, framed by well-developed hypotheses and backed by a solid experimental design.

Indeed your data does add new knowledge to a growing pool of evidence that soil moisture dynamics may moderate the soil C loss through anaerobic processes co-occurring in otherwise upland or drained minerogenic soils. Especially, addition of CH4 dynamics as an integral component of C loss under rewetting is in my opinion a major strength of your manuscript and linking it to the biogeochemistry of Fe dynamics points a way forward for field testing and development of models.

I have added my review as an annotated file.

I have two minor issues though I believe you should address in more detail in the introduction and discussion

1. Period of redox change/flooding

You should discuss in more detail what the effect on C mineralization is of shorter periods of redox changes than the 82 days you are using. What is the argument for 82 days? In my opinion the moisture manipulation period is a major factor and an important dynamics to consider when scaling these ideas to a potential field study. In a natural setting, yes 82 days, may be realistic for sustained high moisture content in depression and footslope soils as you simulate here, but is this the case for artificially drained soils or even naturally in the ridge soils? Thus, how representative is 82 days compared to field observations of soil moisture in this landscape - considering also artificially drained soils? Looking at Fig 1 the effect of soil moisture become less and less important a short intervals which is not surprising. So to maximize impact of your study I do believe a more thorough discussion of the flooding period is needed.

2. Soil types and hydrological regime

Convincingly, your results does indicate that added C loss is mainly driven by hydrology and less by soil type. However, this may be because the soils are relatively similar. They are rich in clay, which promotes protection and complexing of organic C with Fe-oxides and allows to sustain high levels of WFPS. How would your results turn out if the same study was carried out in soils with a coarser texture, e.g. more sand and less clay? This would also alter the hydrological regime with shorter
periods of flooding under field conditions (see comment above). Translating your findings in to an operational routine for a model would need to consider the role of soil texture as well as this may constrain rates. In my opinion this is also important to discuss if you want your results to connect to a broader audience.

That being said, your study in isolation makes up for a very good study as is, but you can increase the value by including the abovementioned points.

Reviewer #2 (Remarks to the Author):

The manuscript by Huang & Hall challenges our current understanding that elevated soil moisture and associated anaerobic conditions suppress soil organic C mineralization. Results indicate that under reducing conditions there is even a greater C mineralization than under aerobic ones. As the lost C is old and very likely result from a dissolution of stable mineral associated organic matter, the identified processes may have a great impact on soil’s C balance. The relation of soil organic matter and moisture in moister soils is an emerging and so far broadly overlooked topic and the manuscript makes a novel and interesting contribution to it. It may stimulate further research on the duration and susceptibility of SOM stabilization by Fe-oxides.

While soil C models currently apply the concept that C mineralization decreases with water saturation, the relative high C release observed by huang & Hall could also be deduced from various other studies. For instance, there are a number of studies showing an enhanced DOC release under reducing conditions. Also, Hanke et al (2013, European Journal of Soil Science) found that paddy soils lost more C and substantially older C by soil respiratory processes when they became anaerobic. However, although the findings and the ideas are not entirely novel, Huang & Hall present the first thorough assessment including CO2 and CH4 production, stable isotopes, redox potentials, released Fe (II), which will receive a high attention by modeler and experimentalists. Overall, the paper is also nicely written and well set in a theoretical framework.

My main concerns are:

1. The sampling and the set-up of the incubation experiment are somewhat unclear (or sloppy; see more detailed comments below). For instance, it is unclear how large the field was, where the samples had been taken from, if all cores sampled in the field had been mixed to one composite sample and then split for the incubation experiment. This would definitely affect the statistical analysis as there might be no real (field) replicates, but it doesn’t affect the story and the mechanisms identified but of course, the quality of the experiment.

2. The δ13C value in DOC seems unrealistic and greater than any value reported so far (see specific comments). Also the method how DOC was obtained is unclear.
3. The longer-term perspectives should be discussed more thoroughly. Why do anaerobic soils generally have greater SOC stocks when SOC apparently decompose more rapidly or similarly? See discussion about paddy soils.

Specific comments

Introduction

L. 46 mineral associated C. ‘decompose’ over decadal times scale. Her I would rather use the term ‘turn over’ because at the same time, there will be a C input to mineral associated C and thus a ‘replacement’.

Discussion

L. 219 and Figure 4: A δ13C value in DOC of -2 ‰ seems very low and seems to be unique (?) to me. Even in the case that all the DOC is released from C4-derived C, then an enrichment by 14 ‰ as compared to the parent material is extraordinary high. Usually, δ13C values in DOC are not very different from its parent material (0 to 2 ‰), microbial biomass can be enriched in 13C by 4-6 ‰ (e.g. Kaiser et al., 2001; European Journal of Soil Science). Also clarify that you have measured the δ13C in bioavailable DOC and not in total DOC.

L. 229-232 The fact that C3-C appeared to be preferentially associated with Fe oxide phases was intriguing, and could potentially be explained by ecohydrological differences among corn and soybeans. Generally, the average age of mineral-associated C is rather old (>100 y). Therefore, I would not relate it to the different crop types but to the inherent age of the fraction. I suppose that in pre-agricultural time, the area was covered by C3-vegetation (?). Looking at the δ13C value in DOC released by Fe-reduction, it is still strongly enriched in 13C with δ13C values > -20 ‰.

L. 238 ff Refer to Hanke et al. (2013, European Journal of Soil Science) observing an increased 14C age of soil-respired CO2 with water-logging in paddy soils.

Methods:

1. The sampling and the processing of the samples in the lab is rather unclear. How large was the field? How many soil cores were taken to have ‘spatially representative samples’? Have all cores sampled in the field mixed to one composite sample and then split for the incubation experiment or have the soil from the cores been used directly for the incubation? To me it seems that the set-up seems to be pseudo-replicated as the samples were taken from one field per site (soil type) only and lab replicates have been incubated and analyzed. This does not change the mechanism identified, but at least site (here called soil) effects cannot be analyzed and interpreted in a statistical sense...

2. I think it is necessary to show the δ13C measured in soil-respired CO2 and CH4 (in a Figure/Table or Text in the supplemental materials). The authors refer to another publication that
δ13C values of soil-respired CO2 is similar to their bulk sources (L. 361). I do not see why this cannot be shown with own data measured (it should!). Also clarify how you calculated the error of your estimates for C3 vs. C4 contribution?

3. L. 374 Concentrations of DOC. How did you extract DOC? Was it filtered? Did you shake the soil?

4. L. 376 Sources of DOC. Clarify that only the sources of biodegradable DOC was measured. Frequently, this is only a fraction of DOC (see Kalbitz et al., 2003, Geoderma). How much was it in this study?

Figures:

Overall, the quality of the graphs could be improved

1. Axis-scale: use rounded numbers (0, 50, 100, ... not 0, 80, 160,...)

2. Report C mineralization in relation to g SOC not g dry soil to allow the reader to get an estimate of how much (which fraction) has been mineralized (without doing the calculation by himself). In this sense, I would also use the similar units in the numerator and denominator (mg CO2-C/gSOC as in most SOC studies or all in mol).

3. The lines look rather messy. Please try to choose thinner lines, smaller symbols or to improve the clarity of the graphs.

Frank Hagedorn

Reviewer #3 (Remarks to the Author):

General comment:

The paper “Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter” by Wenjuan Huang & Steven J. Hall presents an experimental study of the carbon (C) loss from mineral soils due to elevated moisture (with soils collected from C-rich former grassland and wetland soils from Iowa, USA). The major claim of the paper is that redox fluctuations can accelerate C loss in mineral soils by facilitating microbial access to occluded C. As a consequence, C mineralization increases at elevated soil moisture.
The paper is concise, clear, well written, and of broad interest for the soil biogeochemistry community. The outcome of this investigation sheds new light into the C-Fe cycles. The fact that redox fluctuations can accelerate C loss in mineral soils by facilitating microbial access to occlude C is novel and can stimulate further research efforts in this direction.

The introduction is clear, however this section can be updated to include works previously done on oxygen limitations. As an example, see the work performed by the group of Porporato. The statistical analyses are appropriate and the experiments are in general well designed, despite some details can be further clarified. For instance, it is not clear which were the redox/oxygen conditions of the saturated samples after they were allowed to drain and how the drainage phase affected the Fe-reduction and C-mineralization processes. Also, the authors did not include information on how the water was added to the soils samples in order to maintain the soil moisture at a constant level, nor the soil moisture values during the drainage phase of the saturated samples. From Figure 2, it seems that the saturated samples drained to a soil moisture value of 0.8, so it did not reach the field capacity during the 70 days of drainage. If this is the case, it would be useful to add this information to the text and provide a desaturation curve of these samples in the supplementary information. Furthermore, more information is needed to clarify some experimental choices. For instance, the authors should explain why the intermediate soils were not allowed to drain, or why some analyses were limited to the footslope soils. Finally, this work is not strictly challenging the relationship between soil moisture and C mineralization found by previous authors, rather it extends this relationship to long-term incubation experiments in which the redox conditions promote the losses of protected C. The authors should add further information on the soil types and biogeochemical conditions for which the proposed C losses are expected to be significant.

Suggestions:

1. Add a scheme of the Fe-C processes described in the supplementary information. Although the authors are not focusing on rates and specific mechanisms, a scheme will help the reader to follow the discussion section.

2. Add the Eh and soil moisture values from day 82 to 152 in the saturated/drained soils.

3. Please, provide further information in the methodological section on how the water content was monitored and how the water content of the samples was adjusted. More details are needed.

Specific comments:

Abstract:

Line 16: ‘altered moisture regime’ suggests a change in moisture levels, not necessarily an increase. I would rather use ‘near saturated conditions’ levels or ‘under elevated moisture regimes’.
Line 21: ‘with recent C4-C inputs little affected’ Why were the C4-C inputs little affected? The authors should articulate this point better in the discussion section.

Line 23 ‘periodic increases in moisture’ I would rephrase this sentence given that the loss of C related to iron (Fe) reduction was mainly associated with saturated samples and after a long period of incubation (i.e., 25 days).

Introduction:

Line 27: Please add a reference to support this statement, such as reference [1] of the bibliography provided below (which is already included in the reference list).

Lines 41 and 261-263: This statement is not accurate. Please look at the work performed by Porporato and coauthors [2]. Reference [2] investigates oxygen limitation at elevated soil moisture and quantify the anaerobic respiration rates associated to denitrification. Therefore this citation is relevant.

Lines 49-50: Cite [3] which provides a review of the iron availability in soils.

Line 54: At line 47, the authors state the turnover of mineral-associated C in surface soils is over decadal timescales. However, the authors then say that the release of protected C happens on the weekly to monthly timescales. Please clarify this point.

Line 59 Cite [4] that looked at Iron (III) reduction and phosphorus solubilization in humid tropical forest soils.

Results:

Line 99 and 103: ‘Over the first 25 days’ Figure 1a displays similar values of CO2 around day 15, regardless of the treatments. An enlargement of the first 30 days in the SI would help the reader to better follow the result and discussion section.
Line 105 ‘CO2 production from the footslope soil was equivalent among the three moisture treatments’. This is not clear from Figure 1a.

Line 110 Explain why the intermediate soil samples were not allowed to slowly drain as the saturated ones. This clarification needs to be included in the paper.

Line 110: It is not clear whether the saturated soils were drained to field capacity? Were the saturated/drained soils aerobic after day 82? No information on changes in oxygen concentration or Eh is provided.

Line 118 Which is the reason of the secondary peak in CO2 measured at day 30 for both the intermediate treatment and the control? May this increase be associated with the manual addition of water?

Line 125. ‘Methane emissions....were negligible (< 0.2 % of total C mineralization) in the control’ This result is expected due to the aerobic conditions of the control.

Line 155 ‘However, after 82 days, the cumulative C4-derived C mineralization was significantly higher in the saturated treatment (p < 0.05) and the intermediate treatment (p < 0.01) than in the control’ The control of the footslope soil in Figure 3a seems to show values of mineralized C4-C that are comparable to ones the saturated treatment.

Line 178 The drop observed in Eh for both the intermediate and saturated treatments is in line with the previous comment on Figure 1a.

Line 175: Why did the authors limit the Figure 1 of the Supplementary information to 75 days and did not report the changes in Eh following the drainage of the saturated soils?

Line 185 Why did the authors conduct the companion experiment only on the footslope soils?

Line 176 Why not measure directly oxygen?
Which were the oxygen levels in the saturated soils after day 82 (when the soils were allowed to drain)? There are no changes after day 82 in Eh values for the saturated soil, please explain why the redox values did not increase during the drainage phase.

Is the lower concentration of Fe(II) for the saturated treatment a consequence of the drainage?

Is the DOC in Figure 4b due to C release from C-Fe associations?

This work is not challenging the relationship between soil moisture and C mineralization found by previous authors (e.g., reference [5]), rather it extends their finding to include long-term incubations for which the redox conditions promote the mineralization of protected C.

For the saturated soils, there would be also an advective flux of oxygen due to the drainage of the samples. Please add this information for the sake of physical rigor.

Is Figure3 solely accounting for the CO2 produced by heterotrophic respiration or also for the oxidation of methane? Did the authors quantify the methane oxidation rates for the saturated/drained samples after day 82?

Please quantify the lag at which the C release due to Fe-mediated processes starts. Is the lag associated with a specific range of redox or oxygen conditions?

Were the ground corn leaves added to analyze the consumption of readily C source and compare it to the mineralization of the occluded C source? Please clarify this point
Figures

Figure 1

Please add an enlargement in Figure 1a (or an extra figure in the Supplementary information) of the first 25 days of the experiment.

Figure 4

In Figure 4 the lag time seems of 12 days. Is this lag consistent with the CO2 production observed Figure 1? An enlargement of Figure 1 would help the reader.

References

[1] Skopp, J., Jawson, M. D., & Doran, J. W. (1990). Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal, 54(6), 1619-1625.

[2] Rubol, S., Manzoni, S., Bellin, A., & Porporato, A. (2013). Modeling soil moisture and oxygen effects on soil biogeochemical cycles including dissimilatory nitrate reduction to ammonium (DNRA). Advances in Water Resources, 62, 106-124.

[3] Colombo, C., Palumbo, G., He, J. Z., Pinton, R., & Cesco, S. (2014). Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. Journal of Soils and Sediments, 14(3), 538-548.

[4] Peretyazhko, T., & Sposito, G. (2005). Iron (III) reduction and phosphorous solubilization in humid tropical forest soils. Geochimica et Cosmochimica Acta, 69(14), 3643-3652.

[5] Linn, D. M. & Doran, J. W. Effect of water-filled pore-sapce on carbon-dioxide and nitrous-oxide production in tilled and nontilled soils. Soil Sci. Soc. AM. J. 48, 1267-1272 (1984)
Reviewers' comments:

Reviewer #1 (Remarks to the Author):

(1) Period of redox change/flooding
You should discuss in more detail what the effect on C mineralization is of shorter periods of redox changes than the 82 days you are using. What is the argument for 82 days? In my opinion the moisture manipulation period is a major factor and an important dynamics to consider when scaling these ideas to a potential field study. In a natural setting, yes 82 days, may be realistic for sustained high moisture content in depression and footslope soils as you simulate here, but is this the case for artificially drained soils or even naturally in the ridge soils? Thus, how representative is 82 days compared to field observations of soil moisture in this landscape - considering also artificially drained soils? Looking at Fig 1 the effect of soil moisture become less and less important a short intervals which is not surprising. So to maximize impact of your study I do believe a more thorough discussion of the flooding period is needed.

Response: Agreed. We have added more information on why the soils were saturated for 82 days in the revised text in several places, and cited data from regional wetland hydroperiods in van der Valk 2005:

Lines 81-86: “In the most poorly drained soils [in our region], moisture often increases in spring, remains high for several months, and then decreases in mid-summer due to increased evapotranspiration. Although soils on footslopes and depressions are more prone to periodic flooding, soils on ridges also experience seasonal fluctuations in surface moisture and water table depth.”

Lines 96-98: “Saturated soils are allowed to drain slowly after 82 days, analogous to the hydroperiod of seasonal wetlands in our region, while the field capacity and intermediate treatments remain static”

As stated in the new text, we chose 82 days because it is representative of seasonal periods of saturation (spring – early summer) that commonly occur in the most poorly drained soils in our region, and which occurred to an even greater extent prior to European settlement and drainage infrastructure installation (van der Valk 2005).

The extended period of saturation and drainage provides a useful end member for challenging the traditional moisture response relationship of soil C mineralization, which was the focus of this particular study. We agree that shorter-term fluctuations are also of interest, and these are the focus of ongoing work in our research group. We have added the following text as justification (lines 98-101):

“Shorter-term moisture fluctuations (days) are also of interest in these ecosystems, but here we seek to assess biogeochemical impacts of elevated moisture over weeks – months as an end member to challenge conceptual models of heterotrophic activity
developed over shorter timescales.”

Furthermore, we wanted to use a period of flooding that was long enough to achieve pseudo-steady state Eh and trace gas emissions to use as an end-member for comparison with the other treatments. We note, however, that our high-frequency gas flux measurements allowed us to explore the temporal dynamics of microbial responses to saturation over the entire period of 82 days.

References:
van der Valk, A. G. Water-level fluctuations in North American prairie wetlands. Hydrobiologia 539, 171-188 (2005)
Khan, F. A. & Fenton, T. E. Saturated zones and soil morphology in a Mollisol catena of central Iowa. Soil Sci. Soc. Am. J. 58, 1457-1464 (1994)

(2) Soil types and hydrological regime
Convincingly, your results do indicate that added C loss is mainly driven by hydrology and less by soil type. However, this may be because the soils are relatively similar: They are rich in clay, which promotes protection and complexing of organic C with Fe-oxides and allows to sustain high levels of WFPS. How would your results turn out if the same study was carried out in soils with a coarser texture, e.g. more sand and less clay? This would also alter the hydrological regime with shorter periods of flooding under field conditions (see comment above). Translating your findings in to an operational routine for a model would need to consider the role of soil texture as well as this may constrain rates. In my opinion this is also important to discuss if you want your results to connect to a broader audience.

Response: Agreed. As suggested, we have added more discussion on how the patterns of C mineralization under elevated moisture observed in our study might vary in soils with a coarser texture:

Lines 340-347: “The importance of elevated moisture in driving Fe-mediated C release will also likely vary as a function of soil characteristics, especially texture. For example, well-drained coarse-textured soils may seldom experience soil saturation and reducing conditions even under substantially elevated precipitation inputs, although this topic merits further study. The clay-rich soils in our study system represent an intermediate between traditional wetlands and well-drained uplands, where Fe-associated organic matter may respond dynamically to redox cycling. We note that similar hydric soils are prevalent across a broad portion of the North American Corn Belt, a region with historically large soil C stocks49,50,”

Abstract
(3) Line 17 How much above?
Response: Unfortunately, to meet the abstract word limit (150 words) we could not provide details in the abstract about the WFPS values and their variation over time in
the saturated/drained treatment. We think that the key point for the abstract is that we observed reducing conditions in both of the treatments with moisture above field capacity. We have clearly specified the three moisture levels in the last paragraph of the Introduction (Lines 94-98), as well as in the methods:

“In this study, we assess the effects of soil moisture on soil CO2 and CH4 production and δ13C composition in three topographic positions (ridge, footslope, depression) at three soil moisture levels: field capacity, intermediate, and saturation (51, 77, and 99% water-filled pore space (WFPS), respectively). Saturated soils are allowed to drain slowly after 82 days, analogous to the hydroperiod of seasonal wetlands in our region, while the field capacity and intermediate treatments remain static.”.

(4) Line 20 Please indicate in which measure? Was it on accumulated gaseous C-loss in weight over the 25 day period or comparison of instantaneous CO2 and CH4 fluxes between controls and moisture manipulations.

Response: It was the accumulated gaseous C-loss (mass) over the entire period of this experiment. We have revised “total C mineralization as CO2 and CH4” to “cumulative gaseous C loss as CO2 and CH4” to make it clearer. (Line 20)

(5) Line 20 Just for clarity add that it is carbon isotopes

Response: Agreed. We have revised “stable isotopes” to “stable C isotopes” (Line 20)

Introduction

(6) Line 36 Please make it clear for the reader that this is really diffusion in the soil water. This will make it easier to follow your argumentation below.

Response: Agreed. We have clearly stated that this is C substrate diffusion through soil water in the sentence. The phase “oxygen availability and substrate diffusion” has been revised to “oxygen (O2) supply from the atmosphere and C substrate diffusion through soil water” (Lines 35-36).

(7) Lines 39-41 Still, it would be appropriate with a key ref here addressing this knowledge gap.

Response: Agreed. As this point was also raised by the third Reviewer, we have revised this sentence and added relevant references (Rubol et al. 2013; McNicol & Silver 2014). This sentence was revised to “However, the respiratory response at the other end of the moisture curve, where high moisture limits O2 availability, has received less attention”7,8 (Lines 39-40)

Rubol, S., Manzoni, S., Bellin, A. & Porporato, A. Modeling soil moisture and oxygen effects on soil biogeochemical cycles including dissimilatory nitrate reduction to
ammonium (DNRA). Adv. Water Resour. 62, 106-124 (2013).

McNicol, G. & Silver, W. L. Separate effects of flooding and anaerobiosis on soil greenhouse gas emissions and redox sensitive biogeochemistry. J. Geophys. Res-Biogeosci. 119, 557-566 (2014)

(8) Line 45 Have a look at this recent paper in Biogeosciences by Zhao et al (2016) Iron-bound organic carbon in forest soils: quantification and characterization doi:10.5194/bg-13-4777-2016. It would serve the purpose of your paper to present for the reader the nature of the Fe-bound organic C in more detail. You mention below the word “labile” which is a key concept underlying your assumptions.

Response: Agreed. We have now cited this paper “Zhao et al. (2016)” and added more information on the nature of the Fe-bound organic C. We have added the sentence “For example, hydrophilic and carboxylic C that is readily assimilated by microbes can be stabilized by Fe oxides via sorption and co-precipitation10” in this paragraph. Our data on the bioavailability of DOC that we now report in Supplemental Fig. 11 and report in the Results on 248-252 also supports this point. Zhao, Q., et al. Iron-bound organic carbon in forest soils: quantification and characterization. Biogeosciences 13, 4777-4788 (2016).

(9) Line 48 Be consistent. Is this OM in general or organic C only?

Response: We have changed “mineral-associated organic matter” to “mineral-associated organic C” to make it consistent. (Line 50)

(10) Line 54 Change “protected C” to “organic C bound in Fe-oxide minerals”. You may already here indicate that the proposed mechanism is the breakdown of Fe-org C complexes.

Response: Agreed. As suggested, we have revised “protected C” to “organic C that is bound in association with Fe oxide minerals”. (Line 58)

(11) Lines 58-59 What is significant? Is it the response time, the magnitude or the combination. Would be good for the reader to elucidate more on the refs 10, 18

Response: Good point; here we are referring to the response time. This has been clarified in the revised text by revising this sentence to “Iron reduction can potentially occur rapidly (hours – days) following elevated moisture and/or labile C inputs in many terrestrial soils”. (Lines 62-63)

(12) Line 61 First time DOC is mentioned. Define and clarify above that it is mineralization of mobilized organic C in the soil water phase, e.g. DOC, that is in focus.
Response: Agreed. Both colloidal and dissolved organic carbon (DOC) may be released following Fe reduction (Thompson et al. 2006; Buettner et al. 2014; Hagedorn et al. 2000). So, “Fe-mediated DOC release” was revised to “Fe-mediated release of colloidal or dissolved organic C (DOC)”. (Line 65)

(13) L71 This is an important assumption, as the offset of the upstream limitation you mention may be constrained by the structure/properties of released Fe-bound organic C.

Response: Good point; as suggested above, we have specified earlier that labile (i.e., hydrophilic and carboxylic moieties) organic C may be bound to Fe and subsequently released. Please see also our DOC bioavailability assay data in Supplementary Fig. 11 and reported on lines 248-252.

Please also see the revised text on Lines 43-55: “Reactive soil minerals, and iron (Fe) phases in particular, play a critical role in protecting soil C from microbial decomposition. For example, hydrophilic and carboxylic C that is readily assimilated by microbes can be stabilized by Fe oxides via sorption and co-precipitation. The dominant pools of mineral-associated organic C in many surface soils turn over on decadal timescales and vary with geochemical composition, despite the persistence of smaller pools of that cycle over centennial to millennial timescales. However, the biogeochemical processes that drive the release and subsequent decomposition of mineral-associated organic C have received less attention (for example, see Keiluweit et al. 14) than mineral protection of C9,15. In particular, protective associations between Fe mineral phases and soil organic C may be vulnerable to moisture-sensitive redox dynamics. For example, organic C which was previously protected over extended periods by Fe complexation under aerobic conditions could potentially be released and decomposed following Fe reduction.”

(14) L86 What does this represent? Can you provide pF values or water filled pore space numbers?

Response: We have provided the values of the water filled pore space for the three soil moisture levels: field capacity, intermediate, and saturation (51, 77, and 99% water-filled pore space (WFPS), respectively). (Lines 95-97)

Results

(15) Lines 111-112 It would be in place to mention that this is a general observation. However, considerable temporal variation/fluxuation is seen for all three positions/soils which is higher than for the intermediate and control treatments. I think this should be commented as well.
Response: Agreed. We have revised the sentence to “During this period, the temporal variation of CO₂ production was greater in the saturated/drained treatment than the intermediate treatment and control in all three soils. Overall, there was a slow but consistent increase in CO₂ production in the saturated/drained treatment, while CO₂ production slowly decreased in the intermediate treatment and was stable in the control (Fig. 2a).” (Lines 143-147)

(16) Lines 150-153 Was the control and intermediate similar? Please indicate
Response: As suggested, we have indicated the differences in the cumulative mineralization from C₄-derived C in the revised manuscript. We added the sentence “Cumulative mineralization from C₄-derived C in the intermediate treatment was similar with the control during this period.” in the paragraph. (Lines 192-194)

(17) L153-154 The value of cumulative C₄-C mineralization? This is not evident from Fig 3A...
Response: This was unclear, so we have deleted this sentence.

(18) Lines 193-196 The δ₁³C of DOC started out at +2. What is the reason for this apparent enrichment of DOC?
Response: We note that these values were from rapidly biodegradable DOC, not total DOC. The enrichment could be due to transient dynamics of C substrate accumulation and respiratory kinetic fractionation following flooding. We are presently exploring this phenomenon in greater detail, but as these data may distract from the primary message of the paper, we have elected not to present them in the revised manuscript. We therefore used another more conclusive method in this revised manuscript to measure the C isotope ratio of total DOC (Lines 489-502 of Methods). The δ₁³C values of total DOC ranged from -20‰ to -13‰ for the saturated treatment and from -23 ‰ to -16‰ for the control, as would be expected from a mixture of C₃- and C₄-derived DOC. The results on the δ₁³C values of total DOC have been presented in Supplementary Fig. 10. The C₄-derived DOC and C₃-derived DOC have been shown in Fig. 5 of the main text.

(19) Line 198 Reverse order of sup Fig 2 & 3
Response: Agreed. We have corrected the order of the supplementary figures.

Discussion
(20) Lines 229-237 You should build this speculation by including if the annual rotation between soy and corn has a role to play in the type of org C bound to Fe oxides. So C₃-C sorption/coprecipitation to Fe only occurs under soy bean rotations?
Response: Agreed. We have further discussed how the annual rotation between soybean and corn play a role in the type of organic C bound to Fe oxides. These sentences were revised as follows (Lines 305-316):

“The fact that C3-derived C explained most of the increased C mineralization following Fe reduction under elevated moisture was intriguing, and could potentially be explained by ecohydrological differences among corn and soybeans—the two dominant land covers across the North American Corn Belt. Evapotranspiration is a smaller component of the water budget under soybeans than corn" , leading to increased soil moisture during the soybean cultivation phase. Increased soil moisture likely increases the potential for Fe reduction and oxidation during soybean cultivation, and the potential sorption or co-precipitation of fresh C3-derived C inputs from soybean residues on short-range-ordered Fe(III) phases that form following sequential Fe reduction and oxidation. This hypothesis does not negate the occurrence of Fe redox cycling and Fe-C complex formation during corn cultivation, however, and the impacts of plant functional types and ecohydrological characteristics on Fe-mediated soil C dynamics merit further investigation.”

(21) Lines 246-248 This insight is only possible to get because you include CH4, which represents the anaerobic pathway of org C decomposition. This is worth mentioning

Response: Agreed. We have revised this sentence to “these results demonstrate that the release of biochemically labile C following Fe reduction and subsequent mineralization as CO2 and CH4 can potentially offset kinetic or thermodynamic constraints that have previously been thought to limit decomposition under anaerobic conditions" , even as a significant portion of the soil C or DOC pool may remain protected” (Lines 317-321). Later, we stated that “Omission of CH4 measurements in other studies investigating relationships between moisture and soil C mineralization may have also contributed to differences between our findings and the standard conceptual model (Fig. 3).” (Lines 329-331)

(22) Line 268 organic C

Response: Agreed. We have revised “C” to “organic C”. (Line 358)

(23) Line 269 underestimate gaseous C as CO2 and CH4 losses

Response: Agreed. We have revised “underestimate losses” to “underestimate CO2 and CH4 emissions”. (Line 359)

(24) Lines 271-273 ...through the release of Fe-bound organic C
Response: Agreed. We have revised this sentence to “When Fe reduction occurs (e.g., after as little as two days of elevated moisture), it can accelerate C loss in mineral soils by facilitating microbial access to previously protected labile C”. (Lines 361-363)

(25) Lines 273-275 Yes, if the model would also include CH4 and CO2 processes.

Response: Agreed. We have added “and including CH4 as well as CO2 production” in the sentence. (Lines 365-366)

Methods
(26) L303 “residue”

Response: Agreed. We have corrected “reside” to “residue” in the sentence. (Line 404)

(26) L308 It is not entirely clear to me if this treatment is the field capacity treatment or the field moist conditions as it was under sampling. Please clarify.

Response: This treatment is the field capacity treatment. We have clarified this in the revised manuscript. (Line 411)

(27) Fig. 3 As far as I understand the cumulative mineralization includes both CO2 and CH4. Please clarify this in the caption and figure

Response: Agreed. We have clarified the cumulative mineralization includes both CO2 and CH4 in the caption and figure. Please see Fig. 4.

(28) Fig. 3 Please clarify in the y-axis title that the mineralized C comprises CH4 and CO2.

Response: Agreed. We have clarified that the mineralized C included CH4 and CO2 in the y-axis title. The “Mineralized C4-C” and “Mineralized C3-C” have been revised to “C4-derived CO2 + CH4” and “C3-derived as CO2 + CH4”, respectively. Please see Fig. 4 in the revised manuscript.

Reviewer #2 (Remarks to the Author):

While soil C models currently apply the concept that C mineralization decreases with water saturation, the relative high C release observed by huang & Hall could also be deduced from various other studies. For instance, there are a number of studies showing an enhanced DOC release under reducing conditions. Also, Hanke et al (2013, European Journal of Soil Science) found that paddy soils lost more C and substantially older C by soil respiratory processes when they became anaerobic.
However, although the findings and the ideas are not entirely novel, Huang & Hall present the first thorough assessment including CO2 and CH4 production, stable isotopes, redox potentials, released Fe (II), which will receive a high attention by modeler and experimentalists. Overall, the paper is also nicely written and well set in a theoretical framework.

Response: We really appreciated Dr. Frank Hagedorn’s constructive comments and suggestions. In the first manuscript, we cited many of the earlier studies that showed release of DOC under reducing conditions (e.g., Thompson et al. 2006, Buettner et al. 2014, Hagedorn et al. 2000, Grybos et al. 2009). The key knowledge gap that we identify in this paper and address with our experiments is the apparent contradiction between existing theory, which holds that reducing conditions suppress CO2 production, and this potentially large release of DOC. To our knowledge, none of the previous work in this area has addressed this contradiction. Please see revised text in the Introduction (56-67), which attempts to more clearly drive home this point.

We have added the suggested reference (Hanke et al. 2013) as another important example to discuss the influence of elevated moisture on the mineralization of different C sources. (Lines 302-304).

My main concerns are:
(I) The sampling and the set-up of the incubation experiment are somewhat unclear (or sloppy; see more detailed comments below). For instance, it is unclear how large the field was, where the samples had been taken from, if all cores sampled in the field had been mixed to one composite sample and then split for the incubation experiment. This would definitely affect the statistical analysis as there might be no real (field) replicates, but it doesn’t affect the story and the mechanisms identified but of course, the quality of the experiment.

Response: We have added more information on the sampling and the set-up of the incubation experiment. We have provided a map in the Supplementary Information to show the field sites and the locations of soil sampling (Supplementary Fig. 12). The sampling scheme was intentionally chosen to allow our experiment to focus on moisture treatment effects without being confounded by large core-to-core spatial heterogeneity in C availability, and this is discussed and justified in detail below. Our sampling details and justification are as follows:

Lines 386-398: “We sampled each soil series from each of three separate 200 x 200-m blocks within a ~400 ha field under common management (Supplementary Fig. 12). Six soil cores from each soil series in each block were randomly sampled from 0 – 20 cm (the plow layer) using a 10.2-cm diameter stainless steel auger. Soils from the three blocks were then composited by soil series to generate spatially representative samples at the field scale, which were then split for the incubation experiment. Thus, our incubation samples from each composited soil series are technical replicates
rather than spatial replicates, and inference about these soils cannot necessarily be
extended beyond the area which was sampled. This approach was chosen given that
our focus was to characterize the mean moisture response functions of C
mineralization for these soil map units—as opposed to characterizing fine-scale soil
spatial variation. The high spatial variation in soil C availability and respiration
observed previously in these soils may have otherwise obscured the moisture
impacts we sought to test here.”

(2) The $\delta^{13}C$ value in DOC seems unrealistic and greater than any value reported so
far (see specific comments). Also the method how DOC was obtained is unclear.

Response: We have dealt with this point by conducting additional analyses to
measure $\delta^{13}C$ values of total DOC (lines 489-502 of Methods), as opposed to the
rapidly biodegradable fraction of DOC that was reported in the first manuscript
version. The $\delta^{13}C$ values of total DOC are quite consistent with expected values of
DOC derived from a mixture of C$_3$ and C$_4$ vegetation. The early enrichment of the
bioavailable DOC we reported in the first manuscript could have been due to transient
kinetic fractionation following flooding. We are presently exploring this phenomenon
in greater detail, but think that this point may distract from the primary messages of
the paper, so we have elected to present the more standard $\delta^{13}C$ data from total DOC.

The $\delta^{13}C$ values of total DOC ranged from -20‰ to -13‰ for the saturated treatment
and from -23‰ to -16‰ for the control, as would be expected from a mixture of C$_3$-
and C$_4$-derived DOC. The results on the $\delta^{13}C$ values of total DOC have been
presented in the Supplementary Fig. 10. The C$_4$-derived DOC and C$_3$-derived DOC
have been shown in Fig. 5 of the main text.

The method for measuring $\delta^{13}C$ values of total DOC has been added in the revised
manuscript (Lines 489-502): “The $\delta^{13}C$ values of DOC were assessed by measuring
$\delta^{13}C$ of CO$_2$ produced from oxidation of total DOC by boiling with persulfate.$^{65}$
Specifically, 2 mL aliquots of DOC solution were mixed with 1 mL of the oxidizing
agent (100 mL H$_2$O + 4.9 g K$_2$S$_2$O$_8$ + 200 uL of 85% H$_3$PO$_4$) and then acidified to pH
< 3 with 8.5% H$_3$PO$_4$. The solutions were placed in 20-ml glass vials capped with
teflon septa sealed with aluminum crimps, flushed with CO$_2$-free air for 15 min at 500
mL min$^{-1}$, and heated at 100 °C in a water bath for 60 min. The $\delta^{13}C$ values of CO$_2$
oxidized from DOC solutions were measured on the TGA200A by injection$^{62}$ after
samples cooled to room temperature. Following oxidation, DOC in the solutions was
below detection on the Shimadzu TOC-L, implying quantitative conversion of DOC
to CO$_2$. The CO$_2$ produced from blanks containing the oxidizing agent + deionized
water was also analyzed to correct the sample $\delta^{13}C$ values.”

We have now specified the method for how DOC was obtained: “For DOC
measurements, soil subsamples were extracted by nanopure water in a 1:5
soil:solution mass ratio, shaken for one hour, centrifuged for 10 min at 10,000 rcf, and
then filtered through a pre-combusted 0.7-μm glass fiber filter (Whatman GF-F).”

(Lines 485-487)

(3) The longer-term perspectives should be discussed more thoroughly. Why do anaerobic soils generally have greater SOC stocks when SOC apparently decompose more rapidly or similarly? See discussion about paddy soils.

Response: Agreed, this is an important point. As suggested, the longer-term perspectives have now been discussed more thoroughly in the Discussion section:

Lines 332-346: “In light of our results, one might ask: why is greater SOC content typically observed in anaerobic wetland soils than uplands? The answer may depend on the temporal scales of soil moisture dynamics and the consequent effects on soil C stabilization mechanisms. In consistently flooded soils (i.e., many months – years), the accumulation of particulate organic C due to suppression of anaerobic lignocellulose decay presumably outweighs any loss of Fe-associated labile C pools solubilized following reductive dissolution. Accordingly, in perennially inundated soils, we often observe a greater contribution of particulate vs. mineral-associated C, in contrast to terrestrial soils where mineral-associated C pools often dominate. The importance of elevated moisture in driving Fe-mediated C release will also likely vary as a function of soil characteristics, especially texture. For example, well-drained coarse-textured soils may seldom experience soil saturation and reducing conditions even under substantially elevated precipitation inputs, although this topic merits further study. The clay-rich soils in our study system represent an intermediate between traditional wetlands and well-drained uplands, where Fe-associated organic matter may respond dynamically to redox cycling.”

Specific comments

Introduction

(4) L. 46 mineral associated C. ‘decompose’ over decadal times scale. Her I would rather use the term ‘turn over’ because at the same time, there will be a C input to mineral associated C and thus a ‘replacement’.

Response: Agreed. We have changed “decompose” to “turn over” in the sentence. (Line 47)

Discussion

(5) L. 219 and Figure 4: A δ13C value in DOC of -2 ‰ seems very low and seems to be unique (?) to me. Even in the case that all the DOC is released from C4-derived C, then an enrichment by 14 ‰ as compared to the parent material is extraordinary high. Usually, δ13C values in DOC are not very different from its parent material (0 to 2 ‰), microbial biomass can be enriched in 13C by 4-6 ‰ (e.g. Kaiser et al., 2001; European Journal of Soil Science). Also clarify that you have measured the δ13C in bioavailable DOC and not in total DOC.
Response: Please see the earlier response on lines 476-493 above; we now report δ13C of total DOC for clarity.

(6) L. 229-232 The fact that C3-C appeared to be preferentially associated with Fe oxide phases was intriguing, and could potentially be explained by ecohydrological differences among corn and soybeans. Generally, the average age of mineral-associated C is rather old (>100 y). Therefore, I would not relate it to the different crop types but to the inherent age of the fraction. I suppose that in pre-agricultural time, the area was covered by C3-vegetation (?). Looking at the δ13C value in DOC released by Fe-reduction, it is still strongly enriched in 13C with δ13C values > -20‰.

Response: There is ongoing controversy as to the apparent ages of mineral associated C pools. We respectfully disagree with the reviewer on the point regarding the age distribution of mineral-associated C. While there is indeed a significant amount of passive mineral-associated C that cycles over centennial to millennial timescales, rigorous 14C measurements and modeling have shown that large portions of the mineral-associated C pool cycle on decadal timescales across a wide range of soils (Trumbore et al. 1995; Baisden et al. 2002; Koarashi et al. 2012; Hall et al. 2015). The reported centennial mean turnover times of previous studies may often be an artifact of inappropriate 14C modeling, and the lumping together of mineral-associated C pools with very different turnover times; see more discussion on this point in Hall et al. 2015, Biogeosciences 12, 2471–2487.

Trumbore, S. E., Davidson, E. A., Decamargo, P. B., Nepstad, D. C. & Martinelli, L. A. Belowground cycling of carbon in forests and pastures of eastern Amazonia. Gloa. Biogeochem. Cy. 9, 515-528 (1995).
Baisden, W. T., Amundson, R., Cook, A. C. & Brenner, D. L. Turnover and storage of C and N in five density fractions from California annual grassland surface soils. Glob. Biogeochem. Cy. 16, 1117, doi:10.1029/2001GB001822 (2002)
Koarashi, J., Hockaday, W. C., Masiello, C. A. & Trumbore, S. E. Dynamics of decadal cycling carbon in subsurface soils. J. Geophys. Res. Biogeosciences 117, G03033, doi:10.1029/2012JG002034 (2012)
Hall, S. J., McNicol, G., Natake, T. & Silver, W. L. Large fluxes and rapid turnover of mineral-associated carbon across topographic gradients in a humid tropical forest: insights from paired C-14 analysis. Biogeosciences 12, 2471-2487 (2015)

We have modified the text as follows (Lines 46-48): “The dominant pools of mineral-associated organic C in many surface soils turn over on decadal timescales and vary with geochemical composition, despite the persistence of smaller pools of that cycle over centennial to millennial timescales.”

Therefore, there is not necessarily any a priori expectation about the C3 vs C4
composition of the Fe-associated C, especially given that our site has been under mixed C$_3$ – C$_4$ vegetation for the past several thousand years. We have added clarifying text about the C$_4$ and C$_3$ sources of soil organic C at the site (lines 181-185):

“Soils at our study site supported mixed C$_4$ – C$_3$ prairie and wetland vegetation over the last 10,000 years$^{33}$, and have been cultivated under C$_4$ – C$_3$ crop rotations for at least the past 50 years. For this study, soils were collected following corn harvest and amended with corn residues, such that the most recent C inputs had a C$_4$ isotope signature and C$_3$-derived C was older by at least one year.”

As described above, we have now included the $\delta^{13}$C values of total DOC, as well as a discussion about the factors impacting the $\delta^{13}$C composition of DOC (accumulation of fresh solubilized litter residues in addition to C solubilized following Fe reduction (lines 281-296):

“Isotope mixing models suggested that the DOC accumulated under saturated conditions in the footslope soil was primarily of C$_4$ origin, but that C$_3$-derived DOC also increased relative to the field capacity control (Fig. 5). These data help reconcile our finding of increased C mineralization under elevated moisture with traditional theory, which holds that anaerobic conditions suppress C mineralization due to kinetic and thermodynamic constraints$^{2,3,25,27,39}$. An increase in C$_4$-derived DOC under saturated conditions may partially reflect the accumulation of intermediate decomposition products of C$_4$ litter (added at the beginning of the experiment). This interpretation is consistent with the initial suppression of C$_4$-derived CO$_2$ under saturated conditions that we observed during the first 25 days. Over the ensuing months, however, much of this C$_4$-derived DOC was ultimately mineralized to CO$_2$ and CH$_4$, along with any additional C$_4$-derived DOC mobilized by Fe reduction. Meanwhile, the increase in C$_3$-derived DOC observed under saturated conditions but not the control (Fig. 5) may have fueled the approximately three-fold increase in C$_3$-derived C mineralization that we ultimately observed in the intermediate and saturated/drained treatments relative to the control (Fig. 4).”

(7) L. 238 ff Refer to Hanke et al. (2013, European Journal of Soil Science) observing an increased 14C age of soil-respired CO$_2$ with water-logging in paddy soils.

Response: Agreed, this is very relevant. We have cited this reference as an example to discuss the influence of elevated moisture on the mineralization of different C sources. (Lines 302-304).

Methods:

(8) The sampling and the processing of the samples in the lab is rather unclear. How large was the field? How many soil cores were taken to have ‘spatially representative samples’? Have all cores sampled in the field mixed to one composite sample and
then split for the incubation experiment or have the soil from the cores been used
directly for the incubation? To me it seems that the set-up seems to be
pseudo-replicated as the samples were taken from one field per site (soil type) only
and lab replicates have been incubated and analyzed. This does not change the
mechanism identified, but at least site (here called soil) effects cannot be analyzed
and interpreted in a statistical sense...

Response: According to the reviewer’s suggestion, we have specified the field area
and provided more information and justification for the sampling and the sample
processing in the lab (below). The reviewer is correct that the replicates from each
composite soil series are technical replicates suitable to estimate mean values of a
population, not spatial replicates suitable for assessing spatial variability of a
population. This strategy was chosen to minimize the enormous spatial heterogeneity
in C availability that is evident at the scale of individual soil cores at this site
(Cambardella et al. 1994), and thus increase our capacity to detect impacts of
moisture on C mineralization and Fe dynamics, which were the goals of the present
study (as opposed to characterizing spatial variation in the properties of a given soil
series). We respectfully disagree with the statement that “soil” effects cannot be
analyzed in a statistical sense in our study; rather, the scale of inference must be
appropriately qualified to the scale of the composite samples that were taken and
analyzed within our field (see Hurlbert 1984, Ecological Monographs 54: 187-211).
Each composite soil sample represents the mean of a population of 18 cores sampled
from three spatially separate locations of a given soil series throughout the field. To be
sure, we cannot estimate the spatial variability of that population and generalize to the
landscape, but we can certainly test whether the mean of one population differs from
another mean—analogueous to a block effect in ecology. We have emphasized that our
soil series results cannot be necessarily extended beyond the blocks from which they
were taken.

Cambardella, C. A. et al. Field-scale variability of soil properties in central Iowa soils.
Soil Sci. Soc. Am. J. 58, 1501-1511 (1994).
Hurlbert, S. H. Psedoreplication and the design of Ecological field experiments. Ecol.
Monogr. 54, 187-211 (1984)

Please see lines 386-398: “We sampled each soil series from each of three separate
200 x 200-m blocks within a ~400 ha field under common management
(Supplementary Fig. 12). Six soil cores from each soil series in each block were
randomly sampled from 0 – 20 cm (the plow layer) using a 10.2-cm diameter stainless
steel auger. Soils from the three blocks were then composited by soil series to
generate spatially representative samples at the field scale, which were then split for
the incubation experiment. Thus, our incubation samples from each composited soil
series are technical replicates rather than spatial replicates, and inference about these
soils cannot necessarily be extended beyond the area which was sampled. This
approach was chosen given that our focus was to characterize the mean moisture
response functions of C mineralization for these soil map units—as opposed to
characterizing fine-scale soil spatial variation. The high spatial variation in soil C
availability and respiration observed previously in these soils\textsuperscript{58} may have otherwise
obscured the moisture impacts we sought to test here.”

(9) I think it is necessary to show the $\delta^{13}C$ measured in soil-respired CO$_2$ and CH$_4$
(in a Figure/Table or Text in the supplemental materials).

**Response:** Agreed. As suggested, we have presented the $\delta^{13}C$ values of soil-respired
CO$_2$ and CH$_4$ in the Supplementary Figs 4 and 5.

The authors refer to another publication that $\delta^{13}C$ values of soil-respired CO$_2$ is
similar to their bulk sources (L. 361). I do not see why this cannot be shown with own
data measured (it should!).

We respectfully disagree with the reviewer on this minor point. The main point here is
that there is no strong evidence for systematic fractionation between soil organic C
sources and microbial respiration. Although $\delta^{13}C$ values of soil-respired CO$_2$ have
been shown to be similar to their sources in pure C$_3$ ecosystems (see Breecker et al.
2015 and Hall et al., in press), this is not expected in mixed C$_3$ – C$_4$ systems where C$_3$
and C$_4$ soil organic C have different ages and turnover times. I.e., we would expect C$_4$
C losses to be greater in our study system because of the fresh C$_4$ C that was added at
the beginning of our study.

Please see added text on 469-474: “The time-integrated $\delta^{13}C$ values of soil-respired
CO$_2$ have been shown to be similar to their bulk C sources in pure C$_3$ ecosystems\textsuperscript{61,62},
justifying our use of these end-members. Transient C isotope fractionation during
decomposition may occur, and is likely be expressed in intermediate pools such as
microbial biomass and DOC; however, its overall importance is likely to be minor in
comparison with C$_3$ – C$_4$ isotopic differences\textsuperscript{63}.”

Also clarify how you calculated the error of your estimates for C$_3$ vs. C$_4$
contribution?

We have clarified the original figures by using conventional standard errors for the C$_3$
vs. C$_4$ contributions to C mineralization in the revised manuscript.

(10) L. 374 Concentrations of DOC. How did you extract DOC? Was it filtered? Did
you shake the soil?

Response: As suggested, we have provided more information on the DOC
measurement (Lines 485-487): “For DOC measurements, soil subsamples were
extracted by nanopure water in a 1:5 soil:solution mass ratio, shaken for one hour,
centrifuged for 10 min at 10,000 rcf, and then filtered through a pre-combusted
0.7-μm glass fiber filter (Whatman GF-F).”

(11) L. 376 Sources of DOC. Clarify that only the sources of biodegradable DOC was measured. Frequently, this is only a fraction of DOC (see Kalbitz et al., 2003, Geoderma). How much was it in this study?

Response: Agreed. Now, we have measured both total DOC and the bioavailable fraction. The results on the percentage of readily bioavailable DOC have been shown in the Supplementary Fig. 11. The new text has been added (Lines 251-253): “The mean percentage of readily bioavailable DOC was similar between the control (33 ± 5%) and the saturated treatments (31 ± 5%) (Supplementary Fig. 11).

Figures: Overall, the quality of the graphs could be improved
(12) Axis-scale: use rounded numbers (0, 50, 100, ... not 0, 80, 160,...)

Response: Agreed. We have changed to use rounded numbers in the axis-scale in all the related figures.

(13) Report C mineralization in relation to g SOC not g dry soil to allow the reader to get an estimate of how much (which fraction) has been mineralized (without doing the calculation by himself). In this sense, I would also use the similar units in the numerator and denominator (mg CO2-C/gSOC as in most SOC studies or all in mol).

Response: Agreed, but normalizing by total soil organic C pre-supposes that all soil organic C is equally bioavailable among the three soils, which is not necessarily the case. Here, we see consistent impacts of moisture treatments on soil C mineralization across the three soils when expressed on a mass basis but not a soil C basis, suggesting that the depression soils (with greatest total soil organic C) have a larger slow or passive pool than the other soil types. For full clarity, as suggested, we have added figures of C mineralization normalized by SOC in the Supplementary Information (Supplementary Figs 2 and 6) and used the similar units in the numerator and denominator (mmol C mol⁻¹ SOC) in the related figures.

(14) The lines look rather messy. Please try to choose thinner lines, smaller symbols or to improve the clarity of the graphs.

Response: Agreed. We have changed to use thinner lines and smaller symbols and increased the point size of the axis labels to improve the clarity of the graphs.

Reviewer #3 (Remarks to the Author):

General comment: The introduction is clear, however this section can be updated to include works previously done on oxygen limitations. As an example, see the work performed by the
Response: Good suggestion; we have added the Porporato reference (Rubol et al. 2013) as well as relevant work by McNicol and Silver 2014. (Line 40)

The statistical analyses are appropriate and the experiments are in general well designed, despite some details can be further clarified. For instance, it is not clear which were the redox/oxygen conditions of the saturated samples after they were allowed to drain and how the drainage phase affected the Fe-reduction and C-mineralization processes.

Response: Good point. On the Figures, we have indicated the period of drainage with a vertical dashed line, thus the impact of drainage on CO2 and CH4 fluxes can be assessed visually during this period. We have further discussed trends in emissions following drainage in the text:

“In the saturated/drained treatment, moisture decreased slowly following drainage due to the high clay content of these soils (27–38% clay; Supplementary Table 1). Soil moisture decreased from 99% WFPS under saturated conditions to 76-80% WFPS at the end of the experiment (Supplementary Fig. 3). During this period, the temporal variation of CO2 production was greater in the saturated/drained treatment than the intermediate treatment and control in all three soils. Overall, there was a slow but consistent increase in CO2 production in the saturated/drained treatment, while CO2 production slowly decreased in the intermediate treatment and was stable in the control (Fig. 2a).”

Unfortunately, we do not have Eh data during the drainage period due to a technical problem, but we can extrapolate by comparing Fe(II) and Fe(III) data among treatments at the end of the experiment (Supplemental Fig. 8), where most of the Fe(II) generated under flooded conditions had oxidized following drainage by the end of the experiment (Lines ):

“Fe(II) in the saturated/drained treatment decreased to 11.1 ± 3.7 µmol g⁻¹ by the end of the experiment as a consequence of increased atmospheric O2 from diffusion and advection following drainage, while Fe(II) measured 0.2 ± 0.0 µmol g⁻¹ in the control (Supplementary Fig. 8).”

Also, the authors did not include information on how the water was added to the soils samples in order to maintain the soil moisture at a constant level, nor the soil moisture values during the drainage phase of the saturated samples. From Figure 2, it seems that the saturated samples drained to a soil moisture value of 0.8, so it did not reach the field capacity during the 70 days of drainage. If this is the case, it would be useful to add this information to the text and provide a desaturation curve of these samples in the supplementary information.
Response: Good points: we have added detailed information about how the water was added and maintained in the Methods (Lines 411-417):

“Water was added by injecting a syringe with a stainless steel spinal tap needle to the bottom of the soil core, and slowly moving the needle upward to achieve a uniform distribution of water and displace entrapped air. The initial masses of the soil cores were recorded. Soil moisture was monitored by weighing the mass of the soil cores over three-day intervals throughout the experiment and water was added as necessary to the surface of the soil cores to reach their initial weight to replace evaporation loss.”

We have added the requested figure showing the moisture loss over time following drainage (Supplemental Fig. 3).

Finally, we emphasize that Figure 3 shows the mean moisture content of the saturated/drained treatment during the 152-day experiment, which we have now stated in the figure caption: “Soil moisture for the saturated/drained period at 152 days is the mean value over this experiment.”

Furthermore, more information is needed to clarify some experimental choices. For instance, the authors should explain why the intermediate soils were not allowed to drain, or why some analyses were limited to the footslope soils.

Response: Good point; please also see our response to Reviewer 1 above (Lines 84-122 in this document) about the justification for the moisture treatments. We sought to compare a fluctuating hydroperiod representative of seasonal wetlands in our area with two static hydroperiods (field capacity, and above field capacity but not saturated) to explore the moisture response of C mineralization under static vs. saturated/drained moisture. The sustained intermediate and field capacity treatments provide a useful control for comparison with the saturated/drained treatment.

Lines (97-102): “Saturated soils are allowed to drain slowly after 82 days, analogous to the hydroperiod of seasonal wetlands in our region, while the field capacity and intermediate treatments remain static. Shorter-term moisture fluctuations (days) are also of interest in these ecosystems, but here we seek to assess biogeochemical impacts of elevated moisture over weeks – months as an end member to challenge conceptual models of heterotrophic activity developed over shorter timescales.”

We have now clarified that the additional month-long experiment with the footslope soil was conducted to explore the relationships between Fe reduction, Eh, pH, and DOC at higher resolution with high replication (total n = 96), which required extensive destructive analyses. As this soil had characteristics intermediate between the ridge and depression, we chose it as an illustrative example. We added the
following text (Lines 232-234): “The footslope soil was chosen because it had
hydrological and biogeochemical characteristics (Figs 2 and 4 and Supplementary
Table 1) intermediate between the ridge and depression soils, and similar trends in Eh
following saturation (Supplementary Fig. 7).”

Finally, this work is not strictly challenging the relationship between soil moisture
and C mineralization found by previous authors, rather it extends this relationship to
long-term incubation experiments in which the redox conditions promote the losses of
protected C. The authors should add further information on the soil types and
biogeochemical conditions for which the proposed C losses are expected to be
significant.

Response: This is an excellent point. We have rephrased text in the discussion as
follows, and added context as to where this mechanism might be most important:

(Lines 256-261): “Our results extend the consensus relationship between soil moisture
and C mineralization developed over the last several decades from short-term (days –
weeks) incubations, which showed that increased moisture beyond an optimum level
(typically near field capacity) suppresses microbial respiration2,3,5. We show that this
framework does not necessarily apply over longer timescales, where anaerobic
conditions and Fe reduction stimulated under elevated moisture can promote the
mineralization of protected C (Fig. 3).”

(Lines 332-347): “In light of our results, one might ask: why is greater SOC content
typically observed in anaerobic wetland soils than uplands46? The answer may depend
on the temporal scales of soil moisture dynamics and the consequent effects on soil C
stabilization mechanisms. In consistently flooded soils (i.e., many months – years),
the accumulation of particulate organic C due to suppression of anaerobic
lignocellulose decay27 presumably outweighs any loss of Fe-associated labile C pools
solubilized following reductive dissolution. Accordingly, in perennially inundated
soils, we often observe a greater contribution of particulate vs. mineral-associated C47,
in contrast to terrestrial soils where mineral-associated C pools often dominate48. The
importance of elevated moisture in driving Fe-mediated C release will also likely vary
as a function of soil characteristics, especially texture. For example, well-drained
coarse-textured soils may seldom experience soil saturation and reducing conditions
even under substantially elevated precipitation inputs, although this topic merits
further study. The clay-rich soils in our study system represent an intermediate
between traditional wetlands and well-drained uplands, where Fe-associated organic
matter may respond dynamically to redox cycling. We note that similar hydric soils
are prevalent across a broad portion of the North American Corn Belt, a region with
historically large soil C stocks49,50.”

Suggestions:
(1). Add a scheme of the Fe-C processes described in the supplementary information.
Although the authors are not focusing on rates and specific mechanisms, a scheme will help the reader to follow the discussion section.

**Response:** Agreed. We have added a schematic of the Fe-C processes described in the supplementary information. Please see Supplementary Fig. 1.

(2) Add the Eh and soil moisture values from day 82 to 152 in the saturated/drained soils.

**Response:** Agreed. We have added the soil moisture values from days 82 – 152 (Supplementary Fig. 3). Unfortunately, due to a technical error, we do not have robust Eh data after 75 days. However, as discussed above and shown in Supplementary Fig. 8, Fe data from the end of the experiment demonstrate that most of the Fe(II) pool that accumulated under saturated conditions oxidized following drainage during this period.

(3) Please, provide further information in the methodological section on how the water content was monitored and how the water content of the samples was adjusted. More details are needed.

**Response:** Agreed. Text added as follows:

(Lines 411-417) “Water was added by injecting a syringe with a stainless steel spinal tap needle to the bottom of the soil core, and slowly moving the needle upward to achieve a uniform distribution of water and displace entrapped air. The initial masses of the soil cores were recorded. Soil moisture was monitored by weighing the mass of the soil cores over three-day intervals throughout the experiment and water was added as necessary to the surface of the soil cores to reach their initial weight to replace evaporation loss.”

(Lines 421-424) “Soil moisture in the saturated/drained treatment soil cores was monitored by recording the mass of the soil cores over three-day intervals when adding water to the other two treatments to replace evaporation loss.”

Specific comments: Abstract:

(4) Line 16: ‘altered moisture regime’ suggests a change in moisture levels, not necessarily an increase. I would rather use ‘near saturated conditions’ levels or ‘under elevated moisture regimes’.

**Response:** Agreed. We have changed “altered moisture regime” to “under elevated moisture” (Line 16).

(5) Line 21: ‘with recent C4-C inputs little affected’ Why were the C4-C inputs little affected? The authors should articulate this point better in the discussion section.
Response: Agreed. For clarity, we have removed this point from the Abstract and brought it up in greater detail in the Discussion. One hypothesis that we now articulate in greater detail is that reducing conditions differentially affect the loss of relatively older C (solubilized by Fe reduction) more than newer C, as recently observed by others (Hanke et al. 2013). In our ecosystem, Fe-associated C may have a greater C3 component because of ecohydrological differences, as described in detail on lines 305-316.

(6) Line 23 'periodic increases in moisture' I would rephrase this sentence given that the loss of C related to iron (Fe) reduction was mainly associated with saturated samples and after a long period of incubation (i.e., 25 days).

Response: Agreed; we have rephrased this sentence to “Counter to theory, elevated moisture may significantly accelerate C losses from mineral soils over weeks to months—a critical mechanistic deficiency of current Earth system models”.

Introduction:

(7) Line 27: Please add a reference to support this statement, such as reference [1] of the bibliography provided below (which is already included in the reference list).

Response: Agreed. We have added the reference (Skopp et al. 1990) to support the statement, as well as Linn and Doran 1984.

(8) Lines 41 and 261-263: This statement is not accurate. Please look at the work performed by Porporato and coauthors [2]. Reference [2] investigates oxygen limitation at elevated soil moisture and quantify the anaerobic respiration rates associated to denitrification. Therefore this citation is relevant.

Response: Agreed. We have revised this sentence and added the suggested reference (Rubol et al., 2013) in the statement, as well as McNicol and Silver 2014.

Lines 39-40: “However, the respiratory response at the other end of the moisture curve, where high moisture limits O2 availability, has received less attention (Rubol et al. 2013; McNicol and Silver 2014 )”. We also have cited the suggested reference in the discussion section (Line 352).

(9) Lines 49-50: Cite [3] which provides a review of the iron availability in soils.

Response: Agreed. We have cited the suggested reference (Colombo et al., 2014) at the end of this sentence (Line 51).

(10) Line 54: At line 47, the authors state the turnover of mineral-associated C in surface soils is over decadal timescales. However, the authors then say that the release of protected C happens on the weekly to monthly timescales. Please clarify
Response: Agreed. The decadal turnover times are averages that include some Fe-associated C and some C associated with other mineral phases (see also response to reviewer 2 on Lines 546-574 above). In our conceptual model (Supplemental Figure 1), Fe-complexed C may persist for some time but may rapidly be released and decomposed following Fe reduction. Please see text added on Lines 46-55:

“The dominant pools of mineral-associated organic C in many surface soils turn over on decadal timescales and vary with geochemical composition, despite the persistence of smaller pools of that cycle over centennial to millennial timescales\textsuperscript{11-13}. However, the biogeochemical processes that drive the release and subsequent decomposition of mineral-associated organic C have received less attention (for example, see Keiluweit \textit{et al.}\textsuperscript{14}) than mineral protection of C\textsuperscript{9,15}. In particular, protective associations between Fe mineral phases and soil organic C may be vulnerable to moisture-sensitive redox dynamics\textsuperscript{15-17}. For example, organic C which was previously protected over extended periods by Fe complexation under aerobic conditions could potentially be released and decomposed following Fe reduction.”

\textsuperscript{(11)} Line 59 Cite [4] that looked at Iron (III) reduction and phosphorus solubilization in humid tropical forest soils.

Response: Agreed. We have cited the suggested reference at the end of this sentence.

(Line 63)

Results:

(12) Line 99 and 103: ‘Over the first 25 days ‘Figure 1a displays similar values of CO\textsubscript{2} around day 15, regardless of the treatments. An enlargement of the first 30 days in the SI would help the reader to better follow the result and discussion section.

Response: Agreed. We have added a figure to present the CO\textsubscript{2} production and the cumulative CO\textsubscript{2} over the first 25 days (Fig. 1) in the main text, which would help the reader to better follow the results and discussion section. The sentence has been revised to “During the first 10 days of the experiment, CO\textsubscript{2} production was consistently depressed in the saturated and the intermediate treatments relative to the control in all three soils (p < 0.01 for all three soils) (Fig. 1a)” (Lines 118-121).

(13) Line 105 ‘CO\textsubscript{2} production from the footslope soil was equivalent among the three moisture treatments’. This is not clear from Figure 1a.

Response: As also suggested by the second reviewer, we have used thinner lines and smaller symbols in the figure to make this point clearer. We have clarified that the CO\textsubscript{2} production “became statistically equivalent” during this period (Line 128).
(14) Line 110 Explain why the intermediate soil samples were not allowed to slowly
drain as the saturated ones. This clarification needs to be included in the paper.

Response: Agreed. Please see also the response to reviewer 1. Moisture in the field
capacity and intermediate treatments remained consistent to provide controls relative
to the saturated/drained treatment. We have added the following text:

(Lines 97-99) “Saturated soils are allowed to drain slowly after 82 days, analogous to
the hydroperiod of seasonal wetlands in our region, while the field capacity and
intermediate treatments remain static.”

(Lines 138-139): “Moisture in the field capacity and intermediate treatments remained
consistent to provide controls.”

(15) Line 110: It is not clear whether the saturated soils were drained to field capacity?
Were the saturated/drained soils aerobic after day 82? No information on changes in
oxygen concentration or Eh is provided.

Response: We have provided a figure (Supplementary Fig. 3) to show the changes in
soil moisture in saturated/drained treatment after 82 days in the supplementary
information. The following text has been added: (Line 139-142) “In the
saturated/drained treatment, moisture decreased slowly following drainage due to the
high clay content of these soils (27–38% clay; Supplementary Table 1). Soil moisture
decreased from 99% WFPS under saturated conditions to 76-80% WFPS at the end of
the experiment (Supplementary Fig. 3).”

We did not have any data on Eh after 75 days or the O2 concentration. However, we
have the data on Fe(II) concentrations, which are reflective of O2 limitation. The Fe(II)
data in our results indicate that the Eh increased after drainage of the saturated soils,
but was still lower than the control.

(16) Line 118 Which is the reason of the secondary peak in CO2 measured at day 30
for both the intermediate treatment and the control? May this increase be associated
with the manual addition of water?

Response: We regularly measured CO2 at least two hours after we added the water to
the soil cores. All the treatments were treated in the same way for water addition.
Thus, we do not think the peak in CO2 measured was caused by the manual addition
of water. We speculate that the increase in CO2 measured at the 32nd and 35th days
may have been associated with microbial dynamics and growth on the added C4 litter.
Please note that there is increased variability (larger error bars) and greater production
of C4-derived CO2 during this period. However, we feel that this is speculative and
tangential to the main points of the paper.
(17) Line 125. ‘Methane emissions...were negligible (< 0.2 % of total C mineralization) in the control.’ This result is expected due to the aerobic conditions of the control.

Response: Entirely agreed, but still we felt it was necessary to quantify this. The CH₄ emissions were negligible in the control due to the aerobic conditions. We also added the “due to persistent aerobic conditions” at the end of this sentence to clearly state the results. (Line 158)

(18) Line 155 ‘However, after 82 days, the cumulative C₄-derived C mineralization was significantly higher in the saturated treatment (p < 0.05) and the intermediate treatment (p < 0.01) than in the control.’ The control of the footslope soil in Figure 3a seems to show values of mineralized C₄-C that are comparable to ones the saturated treatment.

Response: Agreed. The difference in the cumulative C₄-derived C mineralization was only significant in the depression soils. We missed “in the depression soils” in the original sentence. Thus, we have corrected this sentence to “at 82 days, the cumulative C₄-derived C mineralization in the depression soil was significantly higher in the saturated and intermediate treatments (p < 0.01 for both) than in the control.” (Lines 194-196)

(19) Line 178 The drop observed in Eh for both the intermediate and saturated treatments is in line with the previous comment on Figure 1a.

Response: This text refers to a drop in Eh under the intermediate and saturated conditions, which is to be expected in the context of O₂ depletion and Fe reduction during this period. It also correlates with decreased CO₂, which fits the conventional model of how soil metabolism responds to anaerobic conditions over the short-term. Please see text added on lines 260-262.

(20) Line 175: Why did the authors limit the Figure 1 of the Supplementary information to 75 days and did not report the changes in Eh following the drainage of the saturated soils?

Response: Unfortunately we do not have robust Eh data after 75 days, due to a technical error. However, we now report trends in moisture for the saturated/drained treatment during this period (Supplemental Fig. 3), as well as reporting the Fe(II) data at the end of the experiment. The Fe(II) concentrations in the saturated/drained treatment at the end of this experiment were much lower than the intermediate treatment, but slightly higher than the control. The Fe(II) data thus indicate the maintenance of reducing conditions in the intermediate treatment, and the oxidation of most Fe(II) produced under saturated conditions following drainage.
21) Line 185 Why did the authors conduct the companion experiment only on the footslope soils?

Response: As noted in the text, the additional month-long experiment with the footslope soil was a case study conducted to explore the relationships between Fe reduction, Eh, pH, and DOC at higher resolution with high replication (total n = 96), which required extensive destructive analyses. As this soil had characteristics intermediate between the ridge and depression, we chose it as an illustrative example. We added the following text: “The footslope soil was chosen because it had hydrological and biogeochemical characteristics (Figs 2 and 4 and Supplementary Table 1) intermediate between the ridge and depression soils, and similar trends in Eh following saturation (Supplementary Fig. 7).” (Lines 232-234)

Due to the expense and labor-intensiveness of this additional experiment, we were limited to conducting it on one soil type.

22) Line 176 Why not measure directly oxygen?

Response: Eh measurements by Pt electrodes are widely used for characterizing the redox status of soils and sediments, and both Eh and reduced Fe are correlated with soil oxygen. Direct measurements of O2 (with electrodes or via gas wells) would have been nice to have, but were not critical to the main goals of our study as set up in the Introduction of the paper.

23) Line 176 Which were the oxygen levels in the saturated soils after day 82 (when the soils were allowed to drain)? There are no changes after day 82 in Eh values for the saturated soil, please explain why the redox values did not increase during the drainage phase.

Response: Eh increased during drainage, as evidenced by the Fe(II) data.

24) Line 182: Is the lower concentration of Fe(II) for the saturated treatment a consequence of the drainage?

Response: Yes, the lower concentration of Fe(II) for the saturated/drained treatment was a consequence of increased atmospheric O2 during the drainage. We have rephrased the sentence to make it clear: Iron reduction continued in the intermediate treatment, and Fe(II) measured 72.0 ± 1.9 µmol g⁻¹ at the end of the experiment (152 days). Fe(II) in the saturated/drained treatment decreased to 11.1 ± 3.7 µmol g⁻¹ by the end of the experiment as a consequence of increased atmospheric O2 from diffusion and advection following drainage, while Fe(II) measured 0.2 ± 0.0 µmol g⁻¹ in the control (Supplementary Fig. 8). (Lines 223-228)

25) Line 191 Is the DOC in Figure 4b due to C release from C-Fe associations?
Response: Yes, at least in part, although accumulation of litter decomposition intermediates also likely contributed. Please see new text on Lines 305-316.

Discussion:
(26) Line 201-203 This work is not challenging the relationship between soil moisture and C mineralization found by previous authors (e.g., reference [5]), rather it extends their finding to include long-term incubations for which the redox conditions promote the mineralization of protected C.

Response: Agreed. We have revised this sentence to (lines 255-260): “Our results extend the consensus relationship between soil moisture and C mineralization developed over the last several decades from short-term (days – weeks) incubations, which showed that increased moisture beyond an optimum level (typically near field capacity) suppresses microbial respiration\textsuperscript{2,3,5}. We show that this framework does not necessarily apply over longer timescales, where anaerobic conditions and Fe reduction stimulated under elevated moisture can promote the mineralization of protected C (Fig. 3).”

(27) Line 250. For the saturated soils, there would be also an advective flux of oxygen due to the drainage of the samples. Please add this information for the sake of physical rigor.

Response: Done; Lines 226-227.

(28) Line 252 Is Figure3 solely accounting for the CO2 produced by heterotrophic respiration or also for the oxidation of methane? Did the authors quantify the methane oxidation rates for the saturated/drained samples after day 82?

Response: Please note that we measured net CO2 and CH4 fluxes, not gross fluxes, during the entire experiment (including days 82-152), as is standard for most incubation studies. We have no explicit quantification of how much of the gross CH4 that was produced within the core may have been oxidized to CO2 prior to efflux, which was beyond the scope of our study. The net fluxes in our study account for both the CO2 produced by heterotrophic respiration and the oxidation of methane. We do quantify the mean contribution of CH4 to total C mineralization (Lines 326-328): “The fractional contribution of CH4 production to total C mineralization in our study (mean of 0.34 in the intermediate and saturated/drained treatments) fell within the reported range of 0.25 - 0.67 in other methanogenic soils\textsuperscript{45}.”

(29) Line 273. Please quantify the lag at which the C release due to Fe-mediated processes starts. Is the lag associated with a specific range of redox or oxygen conditions?
Response: Agreed. We have quantified the lag time at which the C release due to Fe-mediated processes starts. We observe an increase an Fe(II) after as little as two days, accompanied by increased DOC, as indicated in Fig. 5. Please see new text on 361-363: “When Fe reduction occurs (e.g., after as little as two days of elevated moisture), it can accelerate C loss in mineral soils by facilitating microbial access to previously protected labile C.”

We caution to ascribe a specific Eh range to Fe reduction as others have done in the past, given that Eh is a mixed potential and therefore Eh values for Fe reduction may vary among systems.

Methods:
(30) Line 301. Were the ground corn leaves added to analyze the consumption of readily C source and compare it to the mineralization of the occluded C source? Please clarify this point

Response: Yes, the ground corn leaves were added to analyze the mineralization of readily available C source and compare it to the mineralization of older (by at least one year) C.

Please see new text in the Results (Lines 183-185): “For this study, soils were collected following corn harvest and amended with corn residues, such that the most recent C inputs had a C₄ isotope signature and C₃-derived C was older by at least one year;”; and new text in the Methods (Lines 403-406): “We amended soils with finely ground corn leaves (10 mg g⁻¹ dry soil) to mimic typical rates of residue incorporation following corn cultivation, which was harvested immediately prior to sampling. The ground corn leaves, along with corn roots produced during the previous growing season, represent the “newest” inputs of C.”

Figures
(31) Figure 1
Please add an enlargement in Figure 1a (or an extra figure in the Supplementary information) of the first 25 days of the experiment.

Response: Agreed. We have added an extra figure to show CO₂ production for the first 25 days of the experiment in the main text (Fig. 1).

(32) Figure 4
In Figure 4 the lag time seems of 12 days. Is this lag consistent with the CO₂ production observed Figure 1? An enlargement of Figure 1 would help the reader.

Response: We have added an extra figure to show CO₂ production for the first 25 days. The lag time is about 10 days, when the mineralization of the released DOC following Fe reduction is enough to compensate the depressed decomposition of
recent C4-C inputs. This is also consistent with the CO2 production observed in Figure 1a.

References

[1] Skopp, J., Jawson, M. D., & Doran, J. W. (1990). Steady-state aerobic microbial activity as a function of soil water content. Soil Science Society of America Journal, 54(6), 1619-1625.

[2] Rubol, S., Manzoni, S., Bellin, A., & Porporato, A. (2013). Modeling soil moisture and oxygen effects on soil biogeochemical cycles including dissimilatory nitrate reduction to ammonium (DNRA). Advances in Water Resources, 62, 106-124.

[3] Colombo, C., Palumbo, G., He, J. Z., Pinton, R., & Cesco, S. (2014). Review on iron availability in soil: interaction of Fe minerals, plants, and microbes. Journal of Soils and Sediments, 14(3), 538-548.

[4] Peretyazhko, T., & Sposito, G. (2005). Iron (III) reduction and phosphorous solubilization in humid tropical forest soils. Geochimica et Cosmochimica Acta, 69(14), 3643-3652.

[5] Linn, D. M. & Doran, J. W. Effect of water-filled pore-sapce on carbon-dioxide and nitrous-oxide production in tilled and nontilled soils. Soil Sci. Soc. AM. J. 48, 1267-1272 (1984)

Response: We have added all the suggested references in the revised manuscript
REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

Hi there

I believe you have done a great job in reviewing your paper and answered the issues raised by all three reviewers.

I have no further comments

Reviewer #2 (Remarks to the Author):

The authors have carefully addressed all the comments by me and the other reviewers, which considerably improved the manuscript. They have added various additional data (e.g. δ13C in total DOC) and information strengthening the conclusions and clarifying the setting, sampling as well experimental set-up. I also appreciate the in-depth discussion of the implication of the findings for the longer term soil organic carbon dynamics. The results strongly improve our understanding of soil moisture effects beyond the optimal values for C mineralization and they also indicate that models may underestimate CO2 and CH4 emissions in environments that experience redox oscillations. I thus strongly recommend the acceptance of the manuscript

I still have the following minor comments:

L. 70-72: try to condense the sentences about enzymatic activities, reads slightly redundant

L. 95 wording: δ13C composition change into ‘ their δ13C values’

L. 102 -107 these are your main results – why do you place them at the end of the introduction - was this a copy-paste mistake?

Fig 1. is part of Fig. 2 – the Figure could be moved to the Supplementals

Frank Hagedorn
Reviewer #3 (Remarks to the Author):

The paper “Elevated moisture stimulates carbon loss from mineral soils by releasing protected organic matter” by Wenjuan Huang & Steven J. Hall presents an experimental study of the carbon (C) loss from mineral soils due to elevated moisture (with soils collected from C-rich former grassland and wetland soils from Iowa, USA). The major claim of the paper is that redox fluctuations can accelerate C loss in mineral soils by facilitating microbial access to occluded C. As a consequence, C mineralization increases at elevated soil moisture. The paper is concise, clear, well written, and of broad interest for the soil biogeochemistry community.

In the revised version of the paper, the authors addressed the Reviewers’ comments and reworked significantly the text and the figures. As a result, the manuscript improved significantly in both quality and clarity. I just have some additional comments that the authors should be able to address quickly. Details are listed below.

Detailed comments:

Lines 54-55 of Figure 1 in the Supplementary Information read: ‘a significant fraction of soil C may be associated with Fe(III) oxyhydroxides’. Could the authors quantify in the main text how big is (or is expected to be) this fraction in soils and how big this fraction needs to be in order to (potentially) lead to a significant source of anaerobic mineralized C?

Line: 140-141. The compositied samples were adjusted to different saturation (51%, 77%, 90%). Given the high clay content, the saturated/drained samples decreased to 76-80% after 82 days of saturation. Waiting for the saturated/drained samples to reach the field capacity would have provided a more meaningful comparison between the saturated/drained treatment and the field capacity control. Please discuss this point.

Line 259: I would change “where anaerobic conditions” to “where prolonged (but not permanent) anaerobic conditions”

Lines 341: Please add a reference (e.g., Hilllel 1998, [7])
Lines 386-398: Which was the porosity value of the core samples? Could the authors provide additional details on how the composited samples were prepared and compacted? All the information required to replicate the experiments should be included in the manuscript.

351-355: This sentence needs editing. Models working on a time scale equal/greater than one described in the manuscript (as [53]) may underestimate the anaerobic release of C when soils are subjected to intermediate hydro period (and a significant fraction of soil C is associated with Fe(III) oxyhydroxide). However, models developed to describe short time scale incubation experiments as [7] are unlikely going to be affected by the anaerobic release of occulted C. This should be carefully clarified in the text.

Can the authors provide the values of the C mineralization rates under aerobic and anaerobic conditions?

Reference:

Hillel, D. (1998). Environmental soil physics: Fundamentals, applications, and environmental considerations. Academic press.
Response to comments: reviewer comments are shown in italic, and our response in regular font

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

Hi there
I believe you have done a great job in reviewing your paper and answered the issues raised by all three reviewers.

I have no further comments

Response: We thank the reviewer for the previous suggestions.

Reviewer #2 (Remarks to the Author):

1. L. 70-72: try to condense the sentences about enzymatic activities, reads slightly redundant

Response: Agreed. We have condensed the sentence about enzymatic activities. The sentences have been revised to “Activities of soil hydrolytic enzymes that proximately control soil organic matter decomposition are thought to decrease under anaerobic conditions because of decreased enzyme production and inhibition from phenolic substances”. Please see Lines 70-72.

2. L. 95 wording: δ13C composition change into ‘ their δ13C values’

Response: Agreed. We have replaced “δ13C composition” to “their δ13C values”. Please see Line 93.

3. L. 102 -107 these are your main results – why do you place them at the end of the introduction - was this a copy-paste mistake?

Response: According to the format requirements of Nature Communications (the last paragraph contains a brief summary of both the results and the conclusions (written in present tense)), we placed our main results at the end of the Introduction.

4. Fig 1. is part of Fig. 2 – the Figure could be moved to the Supplementals

Response: Agreed. We have moved the Fig. 1 to the Supplementary Information (now named Supplementary Fig. 2).
In the revised version of the paper, the authors addressed the Reviewers’ comments and reworked significantly the text and the figures. As a result, the manuscript improved significantly in both quality and clarity. I just have some additional comments that the authors should be able to address quickly. Details are listed below.

Detailed comments:
1. Lines 54-55 of Figure 1 in the Supplementary Information read: ‘a significant fraction of soil C may be associated with Fe(III) oxyhydroxides’. Could the authors quantify in the main text how big is (or is expected to be) this fraction in soils and how big this fraction needs to be in order to (potentially) lead to a significant source of anaerobic mineralized C?

**Response:** Agreed. Previous studies (Wagai and Mayer, 2007; Zhao et al., 2016) have showed that Fe-bound organic C could contribute up to 40% of total organic C in soils. We have cited these two references to indicate how large this fraction of soil organic C associated with Fe(III) oxyhydroxides could be in the main text. Please see Lines 52-54.

2. Line: 140-141. The composited samples were adjusted to different saturation (51%, 77%, 90%). Given the high clay content, the saturated/drained samples decreased to 76-80% after 82 days of saturation. Waiting for the saturated/drained samples to reach the field capacity would have provided a more meaningful comparison between the saturated/drained treatment and the field capacity control. Please discuss this point.

**Response:** This is a relevant point, but our goal here was to assess whether consistent saturation was needed to elicit the observed response in terms of releasing protected C. The saturated/drained treatment C mineralization response shows that even following drainage to intermediate moisture capacity, the net effect on cumulative C mineralization remains the same

3. Line 259: I would change ‘where anaerobic conditions’ to ‘where prolonged (but not permanent) anaerobic conditions”.

**Response:** Agreed. We have changed “where anaerobic conditions” to “where prolonged anaerobic conditions” in the sentence. Please see Line 257.

4. Lines 341: Please add a reference (e.g., Hilllel 1998, [7])

**Response:** Agreed. We have added the suggested reference in the sentence. Please see Line 341.
5. Lines 386-398: Which was the porosity value of the core samples? Could the authors provide additional details on how the composited samples were prepared and compacted? All the information required to replicate the experiments should be included in the manuscript.

**Response:** Agreed. We have added information of the soil porosity of the core samples in the text. We also have provided more additional details on how the composited samples were prepared and compacted (Lines 405-409): Specifically, for each soil core sample, fresh soils (equal to 209.6 g dry soil mass) were mixed well with 2.09 g finely ground corn leaves. The amended soils were then uniformly added to a plastic (butyrate) tube (5 cm diameter, 9.8 cm height) with a polypropylene bottom cap to achieve a bulk density of 1.1 g cm$^{-3}$ and a soil porosity of 58%, representative of field conditions.

6. 351-355: This sentence needs editing. Models working on a time scale equal/greater that then one described in the manuscript (as [53]) may underestimate the anaerobic release of C when soils are subjected to intermediate hydro period (and a significant fraction of soil C is associated with Fe(III) oxyhydroxide). However, models developed to describe short time scale incubation experiments as [7] are unlikely going to be affected by the anaerobic release of occulted C. This should be carefully clarified in the text.

**Response:** We agree with the overall point. On lines 351-356, we have revised this sentence to “Over timescales of days to several weeks, our data closely matched the traditional unimodal relationship between moisture and C mineralization$^{7,52}$. However, over longer timescales, these models may greatly underestimate C mineralization when soils experience sustained periods of elevated moisture, given that Fe reduction has been shown to occur in surface soils spanning a broad spectrum of terrestrial ecosystems$^{22}$.”

7. Can the authors provide the values of the C mineralization rates under aerobic and anaerobic conditions?

**Response:** These data are readily obtainable from the existing Figures in the manuscript and SI, to the degree that such a distinction between strictly aerobic and anaerobic conditions can be made (see below). In our results (Fig. 1 and Supplementary Figs 2, 3), we showed the instantaneous C mineralization (CO$_2$ + CH$_4$) under three soil moisture levels and the corresponding Eh values for these treatments, which represent the degree of anaerobiosis in each treatment. Please note, however, that the words “aerobic” and “anaerobic” are binary terms that do not adequately describe experiment employed here. We have added the following to clarify (318-321): “Analogous to field conditions, soils under intermediate and saturated soil moisture were not isolated from diffusive inputs of atmospheric O$_2$, and contained anaerobic
microsites (reflected by decreased Eh, increased Fe(II), and net positive CH$_4$
emissions) within a partially aerobic matrix.”

8. Reference: Hillel, D. (1998). Environmental soil physics: Fundamentals,
applications, and environmental considerations. Academic press.

Response: This reference has been added in the list of References.