

Response letter-1

General comments:

1. ...For these reasons, it seems problematic to compare N-fixation with N₂O production, and I would ask for an expanded justification for this aspect of the study.

Response: After a careful consideration on the problem, we think it needs further work to build a solid convincing relation between natural BNF and N₂O emissions. Thus, in the revision, we will focus on modeling BNF only.

2. I also think the title is somewhat misleading.....

Response: Because of the adjustment in content, we will give a new title. A tentative title is "Modeling biological nitrogen fixation in global natural terrestrial ecosystems"

3. ...it seems that the model cannot accurately estimate fixation rates for Arctic regions with its current structure leaving me to wonder how to interpret the values reported for Arctic regions?

Response: More observational data in Arctic area will be helpful for a more reliable simulation. Currently, it is not so convincing as that from other ecosystem types. We will try our best to search for more on-site data and related works, for further interpretation of the modeled fixation in Arctic regions.

4. Grammatical mistakes

Response: Thank you for your suggestion. We will correct these.

Minor revisions:

- Ln30: This sentence needs to be tightened up, but the second half could be changed from, 'and decrease from the equator', to 'which decreases from: : :'.
Corrected.

- Ln 34: Remove 'the' prior to 'fixation'.
Corrected.

- Ln 36: Change 'types', to 'type'.
Changed

- Ln 36: Remove 'the' before 'biological nitrogen fixation'.
Changed.

- Ln 51: Gruber and Galloway, 2008, Nature, 451(17), 293-296, would help constrain the quantitative aspect of this sentence.

Thank you for your suggestion. We have added information related to anthropogenic nitrogen into this sentence.

- Ln 80: I think it would help to have a model schematic here - I understand a schematic of the broader model has been published previously, but repeating that schematic and focusing on the newly

integrated processes would help the reader.

Please refer to Figure 1 from Yu and Zhuang (2019). The model schematic is very similar to this one, if not the same.

- Ln 112: What data is required to derive these estimates?

Please refer section 2.3 to the information of data.

- Equation 4: What is the origin of the soil nitrogen inhibition values? Please reference the manuscript these values were taken from.

N_s is soil mineral nitrogen, which is a variable calculated in our model. f_{Nup} is a parameter related to legume nitrogen fixation. Its value and reference manuscript can be found in Table 1.

- Ln 174: I think the authors mean 'a priori values', no?

Yes, it is.

- Ln 226; Should this value be -5 % rather than 5 %? I think the value of -5 % is reported elsewhere in the manuscript.

Thank you for your careful reading. It should be -5% here. we have corrected this part.

- Ln 229: What controls N₂O fluxes within the model?

You can find every detail about the controls of N₂O fluxes from Yu and Zhuang (2019). But actually, we will remove the discussion on N₂O emissions in revision.

- Ln 297: What does 'affected' mean here? It would help to be more specific about direction, for example, does it mean enhanced or reduced?

We have clarified this sentence. It is also explained in the rest of this paragraph.

- Ln 368: Change subheading 'Major controls to: : ':', to, 'Major controls on: : ':'.
Changed

- Seems like Fig. 1 is a repetition of data in the tables? Is Fig. 1 needed? Particularly because there is far more information in the tables.

It is a straightforward visualization with the location information of Table 1. We will consider whether to keep it after the adjustment of text content.

- Please improve the quality of the figure 2, which is quite poor. Why are the x-axis values just floating in the middle of the figure? And why abbreviate them?

We will replot the table. Abbreviations are used here because the original ones are too long.

Technical comments:

- Ln 84: It's not clear to me if the published ARA studies used to calibrate the model have all themselves been calibrated with ¹⁵N measurements? The ARA approach is notoriously difficult to

interpret without reliable calibration, and the conversion of acetylene reduced to nitrogen fixation ranges significantly depending on various factors include the specific nitrogenase enzyme.

We will add a brief introduction about the published methods.

- Ln 124: I'm confused by this temperature relationship, is this a Gaussian distribution similar to that laid out by Houlton et al., (Nature, 2008, 454, 327-330)? It doesn't appear to be - this relationship sounds like there is a very broad plateau whereby temperature does not limit fixation across a wide range (12 - 35 C). Is this correct?

This paragraph gives some examples on the temperature influence on different vegetation types. It is not a Gaussian distribution. We will add some explanation in this part to avoid confusions.

Response letter-2

Response: Thank you for the overall positive feedback. We have thoroughly revised the paper following your comments and suggestions. We find that most problems focused on the relationship between natural N fixation and N₂O emissions. Because it is difficult to avoid the influence of anthropogenic fixed nitrogen on natural ecosystem N₂O emissions at this time. We decide, in the revision, to focus on the simulation of N fixation and eliminate the evaluation of fixation impacts on N₂O emissions.

- 1) The term 'natural' is frequently used throughout the text without attempting to define the context in which it is intended. 'Natural' has many implications, connotations, and hidden assumptions. An explicit definition of the term, as it is used on the paper, is needed. How well do the field sites selected relate to that definition? Indeed, since estimates are that humans have doubled the amount of fixed N applied to the terrestrial landscape of the planet (see the Galloway et al., 2004 reference), how does that relate to the 'natural' sites? More importantly, how can global extrapolation of BNF relate to global N₂O emissions given the substantial contribution that the anthropogenic fixed N (ANF) must have made? While the paper does not completely ignore the importance of ANF, it does not make clear distinctions between the two relative to N₂O emissions.

Response:

- *In this study, we only considered natural ecosystem emissions. Croplands emissions were not modeled. We will add a clear definition of natural in the introduction part.*
- *We cannot guarantee there is absolutely no human effects on selected sites. But according to the cited paper, they are treated as natural environment.*
- *Thank you for your suggestion on ANF's influence on N₂O emissions. We have eliminated N₂O results, and only present the N fixation results.*

- 2) The paper only mentions the TEM and the N₂O emissions model in passing. Little information is provided as to how the BNF model is integrated into those pre-existing models to derive N₂O emissions. Where does the newly fixed N enter into those models? Was it considered to increase the soil organic-N pool size? Or was the assumption made that newly fixed N was all immediately taken up by plants, or both? Were other parameters, such as soil inorganic-N supply, in the previous TEM model modified when the BNF model was included?

Response:

- *We will only simulate the N fixation part with the assistance of TEM.*

- 3) The general conclusion is that including BNF resulted in additional N that led to -5% to +20% changes in seasonal soil N₂O emissions. The main differences occurred in the winter months. That was the range, but what was the central tendency of the effect? Figure 5 suggests that there was generally little change in emissions overall. Indeed, one could easily conclude from Figure 5 that including BNF in the larger model did not have a substantial impact on N₂O emissions. Perhaps that is not too surprising when one

considers that the total fixed N pool size (plant biomass + soil fixed N + atmospheric input) must be substantially larger than the annual amount of newly fixed N from BNF. The abstract states that: “This study highlights that there are relatively large effects of the biological nitrogen fixation on ecosystem nitrogen cycling and soil N₂O emissions.” The results shown in the paper and the discussion do not at all agree with that conclusion relative to N₂O emissions.

Response:

- *We will modify the content of Figure 5.*
- *This study will not highlight the relation between N fixation and N₂O emissions.*

- 4) Given the large overlap in tables and figures between this paper and Yu and Zhuang 2019, one wonders whether the incremental contribution of this paper relative to N₂O emissions represents a publishable, stand-alone contribution over and above Yu and Zhuang 2019. How does this differ from a laboratory experiment that adds little to no new insight into what is already known? In my opinion, the paper can be, and should be, strengthened, by including additional considerations, such as ANF or the differential effect(s) of N speciation on BNF. Or perhaps more radically by reducing or eliminating the focus on N₂O emissions all-together and refocusing on how the BNF inclusion changes the N cycle fluxes in the TEM model. In short, the paper has too much emphasis on N₂O emission given the Yu and Zhuang 2019, paper while more could be done overall concerning the BNF contribution to the model.

Response:

Thank you for your suggestion. We decide to focus our discussion on BNF in the revised version.

- 5) Can N₂O emission data from 8 (line 170), or 6 (line 197), or 5 (Table 3) sites (which is it?) be reasonably extrapolated globally? Those sites were chosen because they were “affected by legumes.” What is the implication of that to the extrapolation? Yes, it was subsequently tested on 35 other (?) sites. But there was little N fixation measured for almost half of those sites

Response:

We will carefully deal with the observational data for N fixation in revision. The N₂O emission data will be removed from the paper.

- 6) Minor revision

- Line 63. The EPA reference is missing.
Added
- Line 104: “and for spatial limitation”. How does that relate to C demand?
Deleted “and for spatial limitation”

- Eqn 1: Nfix is not defined in the text.
Added the definition in the text above Eqn 1.
- Line 143: use the same terminology throughout the paper. Upper threshold is given here. In the table it listed as ‘upper bond’ (sic).
We have changed the “upper bond” to “upper threshold” in the table.
- Line 155: change to read “every unit of nitrogen fixed...”
Added “fixed”.
- Line 161: the units for Cr in the text do not match the units given in Table 1.
Changed the unit in Table 1.
- Line 170: 8 sites are indicated, but only 5 are listed in table 3.
Table 3 will be deleted.
- Line 186 and throughout: use past tense.
Done.
- Line 186: Table 2 lists 7 ecosystem types, not 11.
Changed to “7 ecosystem types among 11”.
- Line 197: Table 3 lists 5 field sites, not 6.
Table 3 will be deleted.
- Line 201: should be “sensitivity testing.”
Done.
- Line 205: Figure 2 is a result, not a method.
Deleted the citation of Figure 2.
- Line 214: no standard deviation is given.
Added.
- Line 214: What is the rationale for “removing these data?”
Because they can be viewed as outliers of observation data.
- Lines 216-217: “simulations are closer : : :in temperate forests: : :” Close to what?
Closer to observation.
- Line 228: There is no N2O data in Figure 3.
We will delete the N2O part.
- Line 244-247: Sentence starting with “Here” is unclear. Is that referring to the

previously cited study or this study? Usually that term refers to the current study, but it appears to be referring to Bruijnzeel et al.

Changed "Here" to "In this study".

- Lines 229-231: "The comparison between measured and simulated data further shows the influence of BNF for different ecosystem types: : ." This is unclear and need further explanation.

This sentence will be deleted.

- Line 248: 32.5 Tg N does not match the number in Table 4

Changed the value in the text.

- Line 248-249: give the numbers in text and refer to Table 4.

Done.

- Line 279-281: This sentence should include some mention of the high nitrate concentrations typically found in desert soils.

We will add a sentence discussing the nitrate concentration in desert.

- Lines 297-298: What was the overall mean and standard deviation of the model results when BNF was and was not included in the N₂O emission simulations?

We will delete the discussion on N₂O emissions.

- Line 374: change slightly to slight.

Changed.

- Lines 381-384: Not all agree with this statement. See Heden et al., 2009, Ann. Rev. Ecol. Evol. Syst. 40:613. Alternate explanations should be included here.

Thank you for providing reference. We will refer to the reference to include other statements.

- Table 1: Change bond to bound throughout. Provide units for all parameters and ranges for coefficients. The description for the Michaelis-Menton constant is incomplete. What process is that a constant for?

We have changed "bond" to "bound" in the description.

Some of coefficients have no unit, but we have added units for those who have.

The Michaelis-Menton constant is for Eqn.5, the consideration of soil carbon.

- Table 2: Column headers should be "measured N fixation rate" and "simulated N fixation rate."

Changed.

- Table 3: Wagga Wagga is in Australia.

Table 3 will be deleted.

- Table 5: N_pot parameter format and units do not match Table 1. What are units for fNup? Units for Kc do not match Table 1.

We have unified the format and units between Table 1, 5 and in the text.

- Figure 1: The grey to blue colors are hard to distinguish against the green and blue background shading. Use the same units for N₂O emissions and N fixation rate here and throughout the paper.

We have unified the units between Figure 1 and text.

- Figure 3: What do the lines represent? Regression lines forced through zero? If so, what is the rationale for doing that?

Yes, the lines are regression lines forced through zero. The inner rationale is explained in the text.

- Figure 5: Point out the y-axis scale differences. The scales chosen for the two tropical forests is rather misleading and are based on what appear to be outlier observations. Suggest using the same scale with a y axis break to include the outliers so that these two panels can be more easily compared. What ecosystem is panel e?

Figure 5 will be removed in revision.

~~Impacts of biological nitrogen fixation on N₂O emissions from global natural terrestrial ecosystem soils: An Analysis with a process-based biogeochemistry model~~ **Modelling**

biological nitrogen fixation in global natural terrestrial ecosystems

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Abstract. Biological nitrogen fixation plays an important role in the global nitrogen cycle. However, the fixation rate has been usually measured or estimated at a particular observational site. To quantify the fixation amount at the global scale, a process-based models ~~are~~ needed. This study develops a biological nitrogen fixation model to quantitatively estimate nitrogen fixation rate by plants in natural environment, and ~~couples it with an extant biogeochemistry model of N₂O emissions to examine the fixation rate and its effects on N₂O emissions. The revised N₂O emission model better matches the observed data in comparison with our previous model that has not considered the fixation effects.~~ The new model estimates that tropical forests have the highest fixation rate among all ecosystem types, which decreases from the equator to the polar region. The estimated nitrogen fixation in global terrestrial ecosystems is 61.5 Tg N yr⁻¹ with a range of 19.8 - 107.9 Tg N yr⁻¹ in the 1990s. Our estimates are relatively low compared to some early estimates using empirical approaches, but comparable to more recent estimates that involve more detailed processes in their modelling. Furthermore, the contribution of nitrogen made by biological nitrogen fixation depends, ~~we estimate that fixation contributes to 5% to 20% changes in N₂O emissions compared to our previous estimates, depending~~ on ecosystem type and climatic conditions. This study highlights that there are relatively large effects of biological nitrogen fixation on ecosystem nitrogen cycling and the large uncertainty of the estimation calls for more comprehensive understanding of biological nitrogen fixation, soil N₂O emissions and calls for more comprehensive understanding of biological nitrogen fixation and More observational data for different ecosystems to improve future quantification of the fixation and its impacts.

1. Introduction

In most terrestrial ecosystems, nitrogen (N) available for plants is generally limited although it is the most abundant element in the atmosphere (LeBauer and Tresder, 2008). ~~H~~-Nitrogen usually enters terrestrial ecosystems through processes of N deposition and from biological N fixation (BNF). Nitrogen deposition is a physical process, representing the direct input of reactive nitrogen ~~(including~~ organic N, ammonia, and nitrogen oxides (NO_y) including nitric oxide (NO), nitrogen dioxide (NO₂), nitric acid (HNO₃) and organic nitrates) from the atmosphere to biosphere. BNF, a biochemical process that converts nonreactive nitrogen (N₂) to reactive nitrogen, provides a liaison between the atmosphere and biological systems. Lightning is also a way to convert N₂, adding 3-5 Tg N yr⁻¹ to terrestrial ecosystems (Levy and Moxim, 1996). Nitrogen input via rock weathering is another important source for terrestrial ecosystems, adding 3-10 kg N ha⁻¹ yr⁻¹ (Morford et al., 2011; Houlton et al., 2018). BNF is significantly greater than lightning induced N fixation (Galloway et al., 1995). On a global scale, anthropogenic nitrogen to the environment could be more than 160 Tg N yr⁻¹ (Gruber and Galloway, 2008), which is even greater than terrestrial N fixation (~110 Tg N yr⁻¹). However, taken together, natural N fixation is the primary source in the absence of human activities to global terrestrial ecosystems. For natural terrestrial ecosystems, the amount of N added is approximately balanced by the nitrogen converted back to the atmosphere (Stedman and Shetter, 1983) and lost into ocean and other aquatic systems.

Once entering terrestrial ecosystems, N can be taken up by plants and microbes, and converted into other oxidized forms through mineralization, nitrification and denitrification. In terrestrial ecosystems, N fixation generally affects the nitrogen cycle and nutrient level to constrain plant productivity. Any change of nitrogen input

to terrestrial ecosystems will influence their soil nitrogen content. ~~N₂O emissions. Atmospheric N₂O absorbs infrared wave and reacts with ozone, affecting the global climate and atmospheric chemical conditions. On a per unit weight basis, N is almost 300 times more effective in trapping heat than carbon dioxide. Its atmospheric concentrations have increased from 286 ppb in 1950 to 327 ppb in 2015 observed in Cape Grim, Australia by the Advanced Global Atmospheric Gases Experiment (AGAGE) Network, and in Alaska, Hawaii and South Pole by National Oceanic and Atmospheric Administration (NOAA). While the increase of concentration is significant due to rising industrial and agricultural emissions, natural ecosystems contribute about 60% of the increase (EPA, 2010). To date, the quantification of the trend and magnitude of N₂O emissions from natural terrestrial ecosystems is still with large uncertainties. Furthermore, the effect of BNF in natural ecosystems on N₂O emissions has not been evaluated.~~

In the process of BNF, N₂ is converted to ammonia by certain soil microorganisms which can then be utilized by and incorporated into plants. In natural environment, N fixation is conducted by two types of microorganisms: asymbiotic organisms, including blue-green algae, lichens and free-living soil bacteria (Belnap, 2002; Granhall & Lid-Torsvik, 1975), and symbiotic organisms, such as fungi and nodule forming Rhizobium species. Among them, the most dominant fixers are leguminous plants and their N fixation mechanisms are also best known (Sullivan et al., 2014; Vitousek et al., 2013). To date, the amount of N fixation by legumes is estimated in the range of 11.3-33.9 kg N ha⁻¹ yr⁻¹ (2.8~8.4 g m⁻² yr⁻¹) in natural terrestrial ecosystems. A symbiotic relationship exists between legume plants and bacteria. In the process, legume plants provide the bacteria energy through photosynthesis, meanwhile, the bacteria around the rhizobia supply the legume N in the form of ammonia.

This study first models the BNF from the symbiotic relationship between legume plants and bacteria, then analyzes factors influencing the fixation rate from different terrestrial ecosystem soils.

~~This study first models the BNF from the symbiotic relationship between legume plants and bacteria. The model is then coupled with an existing N₂O biogeochemistry to quantify the BNF impact on N₂O emissions from global terrestrial ecosystem soils.~~

2. Methods

2.1 Overview

We first develop a BNF model and then couple the model with an earlier version of ~~N₂O~~ biogeochemistry model quantifying soil carbon and nitrogen dynamics (Yu and Zhuang, 2019). The revised model is then used to quantify the BNF at regional and the global scale and its impacts on soil N₂O emissions from in natural terrestrial ecosystems. The BNF rate estimates consider the effects of environmental conditions including temperature, soil moisture, soil mineral nitrogen content and soil carbon content. The new-modified model is calibrated and evaluated with observed N fixation rate data from published studies for various natural terrestrial ecosystems from the Arctic to tropical ecosystems. The model sensitivity to model input is analyzed. The model is then extrapolated to the global terrestrial ecosystems at a monthly step and a spatial resolution of 0.5° by 0.5° for the final decade of the 20th century. The effects of physical conditions on BNF are then analyzed. ~~The effects of BNF and atmospheric nitrogen depositions on N₂O emissions from natural terrestrial ecosystem soils are analyzed.~~

2.2 Model Description

The Terrestrial Ecosystem Model (TEM) is a process-based model that simulates carbon and nitrogen dynamics, hydrological and thermal processes for terrestrial ecosystems. Although many efforts were made to incorporate more details of the N cycle, the N input from the atmosphere to ecosystems has not fully been incorporated to date, especially the BNF as input. Here we improve the N dynamics within TEM by considering N fixation by legumes. The [model schematic and other calculations including of carbon and N fluxes nitrogen cycle and pools](#) are inherited from an earlier version of TEM (Zhuang et al., 2003; Yu and Zhuang, 2019).

BNF is the most significant process in either symbiotic or non-symbiotic forms converting stable molecular N₂ into N chemical compounds that are available to plants. For most terrestrial ecosystems, N fixer could be in many forms, such as free-living bacteria, lichens, and blue algae. But among them, symbiotic BNF is a dominant process to provide biologically accessible N, and most systematical BNF is regulated by legume plants, especially in croplands and semi-natural environment (Mus et al., 2016). In natural environment, contribution from legume can be significant but with large uncertainties, which is greatly determined by various environmental conditions (Lindemann and Glover, 1996). In this study, the N fixation via legume plants is modeled considering (1) the accessible N concentration in soils, (2) the limitation of temperature, (3) soil water status, (4) the carbon demand for N fixation, and [for spatial simulation](#), (5) the percentage of N fixing plants for each ecosystem type as:

$$N_{fix} = N_{fixpot} f_t f_w f_N f_c f_{plant} \quad (1)$$

where [N_{fix} is the nitrogen fixation rate](#), N_{fixpot} is the potential N fixation rate (g N day⁻¹), f_t is the influence function of soil temperature, f_w is the soil water function, f_N is the function of root substrate N concentration, f_c is the function of plant carbon availability, and f_{plant} is the function of legume plant coverage. [Please refer to Table 4 for value range of related parameters.](#)

The potential N fixation is highly related to the total N demand of plants and the available nitrogen in soils. Theoretically, the definition of potential N fixation rate should be the difference between the demand and supply of N. Both of them vary with plant types, stages of growth and soil conditions. For large spatial-scale simulations for various ecosystem types, it is impossible to derive potential N fixation because of data availability. N_{fixpot} can be estimated based on dry matter of root, nodule or plant dry matter (Voisin et al, 2003, 2007). However, root biomass is also difficult to measure directly. In most published studies, the potential nitrogen fixation rate was measured using an acetylene reduction array (ARA) method (Hardy et al, 1968, 1973), and some used ¹⁵N methods (Shearer and Kohl, 1986). In our simulation, N_{fixpot} is assumed to be a constant for each ecosystem type. The N_{fixpot} range is determined from literature and specific values for various ecosystem types are obtained through model parameterization.

Soil temperature is a controlling factor for both microbial activities and plant growth. A large number of studies show that different plants have slightly different preferences for temperature (Montanez et al, 1995; Breitbarth et al., 2007; Gundale et al., 2012). For soybean, 20-35 °C is optimal (Boote et al., 2008), and for white

clover the optimal temperature can be 13-26 °C (Wu and McGechan, 1999). The activity of microbes responds slightly differently to temperature among species. For most of them, the optimum temperature is 20-25 °C, and at 12-35 °C the activity is not limited. Generally, the relation between the factor and temperature is not exactly a Gaussian distribution. BNF increases as the temperature rises from minimum temperature (0-5 °C) for N fixation to optimal temperature, maximum rate occurs within an optimal range (15-25°C), and decreases from optimal to maximum temperature above which BNF will stop at 35-40 °C:

$$f_t = \begin{cases} 0 & \text{when } (t < t_{min} \text{ or } t > t_{max}) \\ \frac{t-t_{min}}{t_{optL}-t_{min}} & \text{when } (t_{min} \leq t < t_{optL}) \\ 1 & \text{when } (t_{optL} \leq t \leq t_{optH}) \\ \frac{t_{max}-t}{t_{max}-t_{optH}} & \text{when } (t_{optH} < t \leq t_{max}) \end{cases} \quad (2)$$

where the upper limit (t_{max}) is set to 45 °C. There is no lower limit, but when t is low enough, f_t will be close to zero (Wu and McGechan, 1999; Boote et al., 2008; Holzworth et al., 2014) (Table 1). For the convenience in computing, a lower limit is set in our model. When the temperature goes beyond its upper or lower limit, f_t is assumed to be 0.

Water stress has a direct effect on nitrogen fixing system (Sprent, 1972). With proper temperature, soil moisture condition is the major factor controlling nitrogen fixation rate (Srivastava and Ambasht, 1994). Soil water deficit and flood dramatically inhibits N fixation because of drought stress and oxygen deficit, respectively (Omari et al., 2004; Mario et al., 2007). In our model, the water factor is linearly related with soil water content (Williams, 1990; Wu and McGachan, 1999):

$$f_w = \begin{cases} 0 & \text{when } (W_f \leq W_a) \\ \varphi_1 + \varphi_2 & \text{when } (W_a < W_f < W_b) \\ 1 & \text{when } (W_f \geq W_b) \end{cases} \quad (3)$$

where W_f ($J \text{ kg}^{-1}$) is the available soil water, which is defined as the ratio of water content to that at the field capacity. In soils, water potential generally includes osmotic and matrix potentials, ranging from -0.1 to -0.3 bar for typical soils, which has little effects on the N fixation. But when the soil gets very dry, the potential can be up to -100 to -200 bar and increases rapidly. W_a is the bottom threshold below which N fixation is totally restricted by soil moisture. W_b is the upper threshold above which nitrogen fixation is not limited by soil moisture. φ_1 and φ_2 are parameters representing the linear relationship between soil water content and its effect on N fixation, respectively (Table 1).

It is generally thought that more substrate N in soils will slow down the N fixation, because plants can uptake N directly from soil with less energy (Vitousek and Field, 1999). By comparison, N fixation needs more energy and consumes more carbon than plant N uptake does. Thus, the N fixation is only considered to occur when the direct N uptake from soil cannot meet the plant N demand. In our model, the inhibition effect of N is defined as (Wu and McGehan, 1999):

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$$f_N = \begin{cases} 1 - f_{Nup} \ln(1000 - N_s) & \text{when } (N_s \geq 0.001) \\ 1 & \text{when } (N_s < 0.001) \end{cases} \quad (4)$$

Where f_{Nup} is a parameter related to legume biological N fixation and soil N. N_s is the soil mineral N (g N m^{-2}). BNF efficiency shows a natural logarithmic relation with the soil mineral N.

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N fixers get photosynthetic carbohydrate support from plants. Because the product of every unit of nitrogen consumes a certain amount of carbon, the lack of carbon supply will inhibit the N fixation. The carbon cost for per unit of fixed N varies widely depending on environmental conditions and ecosystem types. For example, the consumption of carbon is only 1.54 times of fixed N for cowpea (Layzell et al., 1979), and it can be 6.3 to 6.8 times for soybeans (Ryle et al., 1979). It is also related to the life cycle of plants. The carbon effect is modeled following a Michaelis-Menten equation (Boote et al., 1998):

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$$f_c = \frac{1}{1 + K_c / C_r} \quad (5)$$

where C_r is the soil carbon content (g C m^{-2}) to represent carbon availability from plants to N fixers. K_c is the Michaelis-Menten constant, which is plant species dependent.

175 2.3 Data

The classification of land cover and leguminous biomes were derived from the combination of the International Geosphere and Biosphere (IGP) land-cover classification system and the study of Schrire et al (2005). The experimental N_2 fixation data for model calibration were collected for 7 major ecosystem types (Figure 1). Nitrogen fixation rates were determined with acetylene reduction assay (ARA) method in most published studies (Table 2, data were from Cleveland et al. (1999)), expressed in $\text{kg N m}^{-2} \text{yr}^{-1}$. Some of them were measured with the ^{15}N natural abundance technique. ~~Measurements of N_2O emissions affected by legumes were collected at 8 sites, including tropical rainforest in Brazil and Peru, tropical dry forests in Puerto Rico, temperate forest in New York, grassland in Germany and Scotland, and pastures in Australia and Costa Rica (Table 3). Monthly N_2O emission data were organized for comparison with model simulations.~~

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The parameters for N_2 fixation module were initialized with a prior values (Table 2). Ecosystem-specific and microbe guild-specific parameters were inherited from previous TEM model (Zhuang et al., 2003; Yu and Zhuang, 2019). The global simulations were conducted at a spatial resolution of 0.5 by 0.5 degree and at a monthly time step. Historical climate data including temperature, precipitation, cloudiness and water vapor pressure were derived from the Climate Research Unit (CRU) (Mitchell and Jones, 2005). Soil texture data were from Melillo et al. (1993) and Zhuang et al. (2003). Other initial conditions including vegetation properties, soil carbon content and soil nitrogen contents were from Chen and Zhuang (2013) and Zhuang et al. (2012).

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For regional simulations, the total amount of fixed N is also influenced by legume coverage. For each ecosystem type, we estimated the coverage according to the distribution of legume plants and field studies (Table

195 ~~43~~, the coverage data are compiled from Cleveland et al., (1999)), where the minimum and maximum values were derived from the abundance of N-fixers.

2.4 Model calibration and site-level validation

200 Most model parameters are legume-specific or vegetation-specific and are adjusted based on value ranges from previous studies (Table 1). Model is parameterized for ~~11-7~~ representative natural terrestrial ecosystems (Table 2). Root mean square error (RMSE) and coefficient of determination ($0 \leq R^2 \leq 1$) were used for model calibration. RMSE was calculated to show the mean difference between simulated data and observational values. The model is iterated with changing parameters until the RMSE reached a certain value for each site. Most parameters in the model driving nitrification and denitrification have been defined and calibrated in previous studies (Yu and Zhuang, 2019). The calibrated model is evaluated at the site level (~~Table 3~~) and then extrapolated to the global terrestrial ecosystems.

205 ~~To evaluate the model's performance on capturing seasonal trends, the simulated N₂O emissions were plotted along with observational data for the observational period. A linear regression is conducted to show the similarity and discrepancy between simulations and observations, R², along with the slope and intercept of the linear regression line are calculated. Six field observational sites having legume were organized for the model validation (Table 3). Monthly N₂O emission data were used for model comparison.~~

210 2.5 Model Sensitivity and Uncertainty Analysis

The response of N fixation ~~and N₂O emissions~~ of different biomes to input data, and variation of parameters was analyzed using sensitivity test. Four major input variables were selected, including air temperature, precipitation, soil nitrogen content and soil organic carbon content. The monthly average input variables were changed by $\pm 10\%$ of the original level for each site and each grid. The variables were changed at 6 levels, respectively, and the rest of input variables were kept at their original values. The sensitivity was calculated by comparing the simulated annual nitrogen fixation to the simulations with the original input values.

215 ~~The sensitivity was calculated by comparing the simulated annual N₂O emissions and nitrogen fixation to the simulations with the original input values (Figure 2).~~

3. Results

220 3.1 Model evaluation

To evaluate the model, thirty-five observational sites were selected for 7 major ecosystem types across the globe, representing different climate and soil conditions. The experimental data of N fixation have a mean value of $12.9 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, with a standard deviation of $17.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$. The maximum observed fixation occurred in

temperate forest in New Zealand, while the minimum rate was also for temperate forest in Idaho State of the US.

225 Our simulations are comparable with the observed data for all major ecosystem types with the coefficient of
determination (R^2) of 0.44 and with a slope of 0.46 (Figure 32). The regression results are mainly influenced by
some observed data greater than $30 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (~~mean + standard deviation~~). By removing the outliers of
observational~~these~~ data, the slope of regression increases to 0.72. Observational data for temperate forests show the
greatest variation among all major ecosystem types, with a maximum value reaching 800 times of the minimum one.
230 Simulations are closer to the observations across sites in temperate forests with R^2 of 0.26 and slope of 0.42. Our
model underestimated nitrogen fixation rate in temperate forests. The large variation in observations may be due to
the distribution of legume plants, different sampling time periods (e.g., growing and non-growing seasons), and
varying climate conditions. For tropical forests, our model estimates of N fixation are higher than observations with
the slope of 0.75 and R^2 of 0.44.

235 ~~The model was also tested by comparing the simulated N_2O emissions with observed data for 5 sites and 3
ecosystem types including tropical forests, temperate forests, and grasslands. All observed data were converted into
monthly average values. Our model reasonably reproduced the seasonal variations of N_2O emissions at
observational sites. In our earlier version of the model, biological nitrogen input was assumed to be a constant
throughout the year (Yu and Zhuang 2019). Compared to our earlier version, the current version contributes 5% to
240 20% of the total N_2O emission, but only leads to a minor difference to total seasonal trend throughout the year
(Figure 3). There are still some discrepancies between simulated and observed N_2O emissions, which could be due
to the uncertainty in measurement, sudden weather events and changes in soil characteristics. The comparison
between measured and simulated data further shows the influence of BNF for different ecosystem types on N_2O
emissions. The influence is larger in tropical areas, while by percentage, BNF is more important for grasslands in
245 temperate regions.~~

3.2 Model sensitivity analysis

The model sensitivity analysis quantifies the impact of changes in forcing data on nitrogen fixation rate ~~and~~
 ~~N_2O emissions~~. Climate conditions including air temperature and precipitation, and soil characteristics of nitrogen
content and carbon content varied at 3 levels to examine the sensitivity. The response of nitrogen fixation rate ~~and~~
250 ~~N_2O emissions~~ is quantified for each ecosystem type. The sensitivity test was conducted for all observational sites
(Table 2 ~~and Table 3~~). Temperature is the most sensitive variable (Figure 21). ~~Comparing to N_2O emissions,~~
~~N~~nitrogen fixation is more sensitive to the change of all forcing conditions. Increasing soil nitrogen results in a
lower N fixation, but increasing N_2O emissions. Abundant soil nitrogen content inhibits BNF activity, but stimulates
nitrification and denitrification processes.

255 3.3 Biological nitrogen fixation in global terrestrial ecosystems

Tropical forests in South America, Central Africa and South Asia show a wide range of N fixation rate between 1 and 200 kg N ha⁻¹ yr⁻¹ (Bruijnzeel et al, 1991). Here all plants in tropical rainforest are assumed to fix nitrogen and one set of parameters are applied for all tropical forests. The coverage for tropical forests in the landscape was assumed to be 15% (Cleveland et al., 1999), ranging from 5% to 25%. The N₂ fixation rate was estimated to be 18.2 kg N ha⁻¹ yr⁻¹, which is the highest among all vegetation types. Our simulations show that the total fixed nitrogen ranges from 10.8 Tg N yr⁻¹ to 54 Tg N yr⁻¹, with the average value of 32.5 Tg N yr⁻¹ (Table 34). Nitrogen fixation in tropical forests is almost half of the global total amount and a principal contributor of BNF in natural ecosystems. Tropical forests have the largest potential to fix nitrogen given that the optimal temperature and soil moisture for BNF is relatively easy to have under tropical climatic conditions.

265 Temperate forests cover the largest land area from 30°N to 60°N, including temperate coniferous forest, temperate deciduous forest and temperate evergreen forest. Temperate areas have the majority of legumes and many temperate ecosystems are considered to be N limited. Comparing to other ecosystem types in temperate regions, conifers are likely to limit the reproduction of legumes (Wheatley et al, 2010). In general, plant species carrying nitrogen fixers are only distributed in a small percentage of natural temperate forests, like clear-felled areas and pastures (Boring and Swank, 1984). Cleveland et al. (1999) indicated that the legume coverage ranges from 1% to 10% of the land area only. Consequently, our simulations indicate that N fixation by temperate forests was 12.7 kg N ha⁻¹ yr⁻¹. The estimates of the total nitrogen fixation were between 1.9 and 19.14 Tg N yr⁻¹ (Table 4). Nitrogen fixation in temperate areas contributes 12.5% of the global total amount.

275 Savanna covers over a half of African continent, Australia and large areas of South America. It is an important biome in the Southern Hemisphere. There is a great variation in native legume species. Only in humid savanna, legumes may significantly contribute to the increase of soil nitrogen (Cech et al., 2008). On average, 15% of the vegetation in savanna is considered as legume grass and biological nitrogen fixation occurs when precipitation is greater than 10 mm per month. Generally, nitrogen fixation in savanna is restricted by soil moisture, while temperate grassland is limited by both temperature and soil moisture (Bustamante et al, 1970). Nitrogen fixers are not abundant for these biomes (Woodmansee et al., 1981). The coverage of nitrogen fixers was assumed to be from 5% to 25%, (Cleveland et al., 1999). Our simulation assumed that nitrogen fixers cover 15% of the land, resulting in 1.9 kg N ha⁻¹ yr⁻¹ fixation, representing a much smaller fraction compared to forest ecosystems. Total fixed nitrogen in grasslands appeared to range from 0.62 to 3.1 Tg N yr⁻¹, with an average of 1.86 Tg N yr⁻¹. For savanna, the total contribution was less due to its relatively small area. The minimum, average and maximum values were estimated to be 0.45, 1.34 and 2.23 Tg N yr⁻¹, respectively.

285 In tundra and boreal forest regions, both host plants and their rhizobia are adapted to the environment with low temperature. Nitrogen fixation rate is extremely variable for boreal ecosystems. For tundra, the coverage was assumed to be 3-15%, and for boreal forest, the coverage was 4-18%. But in general, the low temperature and permafrost conditions limit the activity of nitrogen fixers (Alexander, 1981). We estimated that tundra ecosystems fix nitrogen at 3.2 kg N ha⁻¹ yr⁻¹. Their total BNF was between 0.51 to 2.55 Tg N yr⁻¹ with average of 1.54 Tg N yr⁻¹. In boreal forests, the fixation rate was much lower (2.1 kg N ha⁻¹ yr⁻¹) compared to temperate forests.

In deserts, although some legumes may exist in extremely dry conditions, and some species may grow rapidly after rainfall, their fixation could be neglected. However, in semi-arid areas, legumes are common plants with several species, their N fixation is lower than tropical and temperate forests ($5.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

295 Mediterranean ecosystems such as in south California and some areas in South Australia are characterized with mild rainy winter and hot dry summer, containing both evergreen and deciduous shrublands, in which nodulated legumes are prominent (Sprent et al., 2017). These legumes are more active in comparatively wet season than in dry season (Sánchez-Díaz, 2001). The ability to fix nitrogen is considered to be one of the most important features that enable legumes and plants to survive under severe environments (Crisp et al., 2004). We estimated that the N
300 fixation rate of these legume species is similar to that in grasslands ($2.7 \text{ kg N ha}^{-1} \text{ yr}^{-1}$).

Spatially, the highest rate of N fixation occurred in the tropical and sub-tropical areas, as a result of proper climate and soil characteristics for fixers (Figure 4a3). N fixation from tropical forests and xeric shrubland contributes to nearly half of the global terrestrial amount (Table 43). A lower N fixation rate was in high latitudes of East China, North America and Europe, which were mainly covered with temperate forests. Compared to tropical
305 areas, N fixation in temperate regions shows a larger variability depending on vegetation types. The spatial variation could be attributed to the distribution of legume plants, in addition to the difference of humidity and temperature conditions. N fixation in temperate regions accounts for 35% of the total fixed N.

~~3.4. Soil N₂O emissions enhanced by biological nitrogen fixation~~

~~The model was run at site level to estimate the role of BNF on N₂O emissions. Our analysis between current model and previous model without N fixation module indicated that N₂O emissions would be affected by 5% to 20% depending on biome types and seasons (Figure 5). In most cases, we got higher estimation of N₂O emissions because BNF process enhances soil N content. The involvement of BNF strengthens the seasonal variation as BNF led to lower emissions in winter and higher emissions in summer.~~

Our model estimated that high BNF rates in growing season is consistent with other regional and global
315 estimates (Cleveland et al., 1999, 2013; Lee and Son, 2005; Lett and Michelsen, 2014). The energetic cost for active N uptake becomes lowest when soil temperature is around 25°C (Fisher et al. 2010). Similarly, our estimates of high BNF rates also occur at similar temperature conditions in spring and summer. The global soil nitrogen mineralization rate was estimated to be 696 Tg N yr^{-1} while 15% of plant N demand was provided by BNF (Cleveland et al., 2013). Our estimates of BNF were lower than the estimates by Cleveland et al. (2013) and fell
320 within 10% of the total soil mineralization rate. This result also indicates that about 10% of the mineralized N was induced by BNF. ~~As mineralized nitrogen is the substrate for subsequent reactions producing N₂O, the additional N input through BNF to terrestrial ecosystems led to the change of N₂O emissions across biomes.~~

During 1990-2000, our simulations show that BNF in natural terrestrial ecosystems is $61.5 \text{ Tg N yr}^{-1}$, but anthropogenic N fixation was much higher at 140 Tg N yr^{-1} (Galloway et al., 2002). This large amount of
325 anthropogenic N input to terrestrial ecosystems is expected to inhibit the natural BNF and might lead to less BNF in the future. ~~Our simulations indicate that BNF has a minor influence on N₂O emissions for most high latitude~~

~~ecosystems and extremely arid regions, as a result of low temperatures and drought (Figure 4b). Overall, the fixation as additional N to soils led to 5% to 20% changes in soil N₂O emissions.~~

4. Discussion

330 4.1 Comparison with other estimates of biological nitrogen fixation

There is a large uncertainty in estimating the N input into terrestrial ecosystems, especially from BNF (Sutton et al., 2014) (Table 43). In our study, a calibrated process-based model was applied to estimate site-level and global BNF in natural terrestrial ecosystems. Empirical models provide reasonable estimation based on relationships between fixation rate and environmental factors (e.g. evapotranspiration) (Cleveland et al, 1999), while process-based approaches consider processes in BNF affected by multiple controlling factors (Fisher et al., 2010; Gerber et al., 2008; Meyerholt et al., 2016). Our estimated BNF in the global terrestrial ecosystems is 61.5 Tg N yr⁻¹ with an uncertainty ranging from 19.8 to 107.9 Tg N yr⁻¹, which is lower than most existing studies. Cleveland et al. (1999) provided a central value of 195 Tg N yr⁻¹ by scaling up field-based experimental data, with a range of 100 - 289 Tg N yr⁻¹. This range represents potential distribution of nitrogen fixation. In reality, N fixation is also affected by climate and soil conditions, making the actual terrestrial BNF smaller than the potential one. In a more recent study of Cleveland et al. (2013), a total of 127.5 Tg N yr⁻¹ was estimated to be related to BNF, based on the relationship between BNF and evapotranspiration (ET). Galloway et al (2002b) also provided several estimates for global BNF. Galloway et al. (2004) further suggested a range of 100 - 290 Tg N yr⁻¹ and implied that the true rate of BNF would be at the low end of this range without large-scale human disturbance. In an earlier study (Galloway et al., 2002b), the mean annual global BNF was estimated to be 89-100 Tg N yr⁻¹. By assuming a steady state between N input to and loss from ecosystems, Vitousek et al. (2013) estimated the BNF to be 58 Tg N yr⁻¹ with a plausible range of 40 - 100 Tg N yr⁻¹, which is similar to our estimates. However, Xu-Ri and Prentice (2017) estimated that the N fixation was about 340 Tg N yr⁻¹ which is almost 5 times larger than our estimates. In their study, BNF was determined by plant N requirement across all biome types.

345 In our estimation, tropical forests significantly contribute to the total BNF, which is up to 18 kg N ha⁻¹yr⁻¹. This result is highly related to the density of leguminous plants, and the physical conditions in tropical areas (Crews, 1999). Our simulated results are comparable to the estimates of symbiotic N₂ fixation from tropical evergreen (5.5-16 kg N ha⁻¹ yr⁻¹) and deciduous forests (7.5-30 kg N ha⁻¹ yr⁻¹) (Reed et al., 2011). Barron et al. (2010) directly measured N₂-fixing root nodules across lowland tropical forests and their observations also showed a large variation among individual trees. For a mature forest matrix, the average value was around 10 kg N ha⁻¹ yr⁻¹, but it could be as high as 200 kg N ha⁻¹ yr⁻¹ for some areas. Cleveland (2013) provided a similar estimate to ours (around 12 kg N ha⁻¹ yr⁻¹), but higher values (20-30 kg N ha⁻¹ yr⁻¹) in their earlier studies (Cleveland et al., 1999). Sullivan et al (2014) analyzed human's impact on tropical N fixation and found, depending on forest ages, fixation was 5.7 kg N ha⁻¹ yr⁻¹ with a range from 1.2 to 14.4 kg N ha⁻¹ yr⁻¹, which is lower than our estimates.

360 For temperate and boreal forests, we estimated that BNF fixation is 2.1-18 kg N ha⁻¹ yr⁻¹. The existing BNF estimates from literature also show a large uncertainty for those forest ecosystems. For instance, LM3V-N model

(Gerber et al., 2009) suggested that the N input to forests to be less than 5 kg N ha⁻¹ yr⁻¹. But their model also estimated that, in moist forests, the uptake of N could be 30-80 kg N ha⁻¹ yr⁻¹. Deluca et al. (2002) reported that cyanobacterium and feather moss could act as a supplement to N fixation in boreal forests (0.5 kg N ha⁻¹ yr⁻¹) while the organic N accumulation could be 3 kg N ha⁻¹ yr⁻¹. For the forests in northwest Rocky Mountain, N fixation amount is on average between 0.5 and 2 kg N ha⁻¹ yr⁻¹ (Clayton and Kennedy, 1985; Fahey et al., 1988) while Kou-Giesbrecht and Menge's model (2019) estimated the N fixation rate to be 0 -10 kg N ha⁻¹ yr⁻¹ for temperate forests, and 0 to 6 kg N ha⁻¹ yr⁻¹ for boreal forests.

There could be a number of reasons for our comparatively lower estimates. The most important one is that there is a considerable uncertainty in estimating the coverage of N fixing plants. High diversity in the distribution of legume plants highly influences the estimation of total plant coverage, because our estimation was based on site-level experimental data. In order to improve our understanding, more investigation on legume plant distribution and associated data for N fixers is needed, especially in the Middle Asia, South America and Africa.

Large variations of BNF rates exist across terrestrial ecosystems spatially (Figure 4a). The global BNF spatial pattern is similar to other estimates (Cleveland et al., 1999; Xu-Ri and Prentice, 2017). The highest N fixation rate in tropical regions (more than 50% of the global terrestrial N fixation) is primarily due to their warm and moist soil conditions. Further, N fixed by human activities became increasingly influential in the past century (Galloway et al., 2002), especially in temperate regions due to their large human population. The anthropogenic N deposition contributed more to soil N than BNF did. As a result, soils became N rich, inhibiting BNF in temperate soils. This could explain why the potential N fixation rate was high in temperate ecosystems, but only contributed to 20% of the total fixation.

4.2 Major controls on biological nitrogen fixation

In our simulations, the N fixation was primarily influenced by soil temperature, moisture and soil nitrogen content. The highest N fixation rate in tropical ecosystems is consistent with our sensitivity analysis for temperature and soil moisture. The sensitivity analysis indicated that a 1-3°C increase of temperature led to 7% increase in N fixation rate. Nitrogen cycle responds differently between different biomes and legume types. But in general, increasing temperature will accelerate processes in the N cycle. Soil moisture correlates with BNF in a similar way with temperature. A slightly increase of precipitation (10%) increased the nitrogenase activity. However, the response of N fixation to soil water stress is not as sensitive as that to the change in temperature. -Xeric shrubland and savanna in dry tropical areas still contribute greatly to the global N fixation, while the contribution of boreal forests, with ~~abundant rainforest and~~ low temperature, is much lower.

BNF is highly regulated by soil nitrogen content. N-deficiency conditions usually favor BNF activities, for example, in xeric shrubland and savanna. Enhancing soil N content will decrease the N fixation rate, which is also consistent with our sensitivity analysis. It costs less energy for plants to take up N directly from soils rather than biologically fixing it from the atmosphere (Cannell and Thornley, 2000). However, there is an exception for some areas in tropical ecosystems. Many tropical soils are comparatively rich in nitrogen, but N-fixing plants are still

active to compensate the nitrogen depletion due to the rapid N cycling (Pons et al., 2007). This explains why N fertilization inhibits the BNF in temperate ecosystems, but BNF is still active in N-rich soils in tropical ecosystems. In areas where the energetic cost exceeds the demand of N, the BNF rate will be comparatively lower. Sullivan et al. (2014) suggested that there were lower rates of BNF in undisturbed mature forests and higher rate in secondary forests, depending on the balance between N-demand and energy consumption.

4.3 Model limitation and future work

The incorporation of BNF into TEM allows us to more adequately estimate N₂O emissions from natural terrestrial ecosystems. However, there are several limitations in this study.

First, the current model ignores the effect of free-living BNF. Although symbiotic BNF is critical for most natural and semi-natural ecosystems, asymbiotic organisms play an important role in extreme environments such as waterlogged soils and deserts. The importance of symbiotic BNF or fixation by leguminous plants may not be as significant as previously thought. Elbert et al. (2012) suggested that cryptogam contributed nearly half BNF in terrestrial ecosystems, which was up to 49 Tg N yr⁻¹. In some tropical areas, the spatial N input from free-living bacteria even exceeds symbiotic input (Sullivan et al., 2014). In addition, legumes are not the only source of symbiotic BNF. Some fungi species have the ability to actively fix atmospheric nitrogen. But in most existing models, fungi or mycorrhizae symbioses are not considered due to the limited knowledge about their mechanisms of fixing N (Fisher et al., 2010). A more comprehensive model that covers various types of nitrogen fixation is needed.

Second, the BNF process in our model is calibrated with a limited amount of data, imposing a general set of parameters to all plant species and soil conditions within an ecosystem type. More observational data from natural terrestrial ecosystems is desirable to improve our model.

Third, it is difficult to isolate the N addition via natural processes from human activities. In the US, 20-35% of annual N input into terrestrial ecosystems are human-related (Sobata et al., 2013). As a result, the quality of observational data varies from site to site, some BNF data are only semi-natural. The observational data are imperfect, which might have also biased our estimates through model parameterization process.

5. Conclusions

This study developed a process-based biological nitrogen fixation model and coupled it with an extant ~~the outliers of observational nitrous oxide~~ emission model. The model was evaluated with observed data for ~~both N fixation and N₂O emissions~~. ~~The model was~~ then extrapolated to the global natural terrestrial ecosystems. Our model estimated that biological nitrogen fixation in natural terrestrial ecosystems was 61.5 Tg N yr⁻¹ during the last decade of the 20th century and the greatest fixation rate occurred in tropical regions. Soil temperature, rather than soil moisture and nutrient content, is the most dominant control to the fixation. ~~We further found that, the fixation as additional N to soils led to 5% to 20% changes in soil N₂O emissions. Both the new and previous models were able to capture the seasonal variation of field observed N₂O, but the new version of the model estimated more seasonal~~

430 ~~dynamics~~. Lacking the knowledge about the distribution of N fixing plants and their physiological features might
have biased our estimates of both biological nitrogen fixation ~~and N₂O emissions~~ at the global scale.

Data availability

435 Climate data including monthly cloudiness, precipitation, temperature, and water vapor pressure are from the
Climate Research Unit (CRU) <http://www.cru.uea.ac.uk/data> (last access: May 2017). Global vegetation data and
soil data are available in Zhuang et al. (2003) and McGuire et al. (2001). The explicit spatial data on soil water pH
from the ORDL gridded soil properties product (https://daac.ornl.gov/cgi-bin/dsvviewer.pl?ds_id=546, last access:
440 March ,2017) are based on the World Inventory of Soil Emission Potentials (WISE) database (Batjes, 2000). The
global average carbon dioxide concentration is observed at NOAA's Mauna Loa Observatory. N deposit data are
from NADP monitor and CASTNET. The initial values of soil microbial carbon and nitrogen, and the ratio of C/ V /
N at the global scale, were from a compilation of global soil microbial biomass carbon, nitrogen, and phosphorus
data (<https://doi.org/10.3334/ORNLDAAAC/1264>, last access: May, 2017). *The data presented in this paper can be
accessed through our research website (<http://www.eaps.purdue.edu/ebdl/>).*

445 Author contribution

Q. Zhuang and T. Yu designed the research. T. Yu performed model simulations and data analysis. Both
authors contributed to the paper writing.

Competing interests

450 The authors declare that they have no conflict of interest.

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Table 1. Description of parameters used in the model

Parameters	Description	Unit	Reference Value	reference
N_fix	nitrogen fixation rate	g N m ⁻² day ⁻¹		
N_fixpot	potential nitrogen fixation rate	g N m ⁻² day ⁻²	0.01-1×10 ⁻³	Thornley (2001); Eckertsten et al.(2006); Corre-Hellou et al. (2007); Corre-Hellou et al. (2009);
ft	soil temperature factor	°C		
t_min	the minimum temperature for the start of N fixation	°C	0.5~5	Boote et al. (2008)
t_max	the maximum temperature for the stop of N fixation	°C	40~45	Boote et al. (2008)
t_optL	lower bound - threshold of optimal temperature	°C	10~20	Boote et al. (2008)
t_optH	upper threshold bound of optimal temperature	°C	25~35	Boote et al. (2008)
fw	soil water factor			
φ1	coefficient for soil moisture		0	
φ2	coefficient for soil moisture		2	APSIM, EPIC (Sharpley and Williams, 1990; Bouniols et al., 1991; Cabelguenne et al., 1999); SOILN (Wu and McGechan, 1999)
Wa	lower threshold bound of water content below which N fixation is totally restrict by rhe deficit of soil water		0	APSIM, EPIC (Sharpley and Williams, 1990; Bouniols et al., 1991; Cabelguenne et al., 1999); SOILN (Wu and McGechan, 1999)
Wb	upper threshold bound of water content above which N fixation is not limited by rhe deficit of soil water		0.5	APSIM, EPIC (Sharpley and Williams, 1990; Bouniols et al., 1991; Cabelguenne et al., 1999); SOILN (Wu and McGechan, 1999)
Wf	available soil water content to that at field capacity			
f_Nup	parameter relating legume biological nitrogen fixation and soil nitrogen content		0.01~0.1	SOILN model (Wu and McGehan, 1999)
Ns	Soil mineral nitrogen content	g N m ⁻²		
f_N	Soil mineral N effect			
f_C	Soil carbon effect			
Cr	Carbon concentration in the soil	g C g ⁻¹ soil		

K_c Michaelis-Menten Constant for carbon $\text{g C m}^{-2} \text{g}^{-1} \text{soil}$ 0.001~0.01 Thornley (2001); Eckertsten et al. (2006)

Table 2. Calibration Sites of Biological Nitrogen Fixation Rate for Representative Ecosystems

Site name	Ecosystem Type	Lon.	Lat.	Experimental method	Reference	N Fixation Rate kg N ha ⁻¹ yr ⁻¹	Simulation, kg N ha ⁻¹ yr ⁻¹
Stordalen, Sweden	Tundra	18	68	ARA	Christie (1987); Sonesson et al. (1980)	2	2.5
Truelove Lowland, Canada	Tundra	-84.5	75.5	ARA	Chapin et al. (1990)	3	2.8
Niwot Ridge, Colorado, US	Tundra	-105.5	40	¹⁵ N	Bowman et al. (1996)	4.9	5.1
Central Sweden	Boreal Forest	18	60	ARA	Nohrstedt (1985)	0.93	0.9
PNFI, Ontario, Canada	Boreal Forest	-77	45.5	ARA	Hendrickson (1990)	0.25	1.2
Southern British Columbia, Canada	Boreal Forest	-119	49	ARA	Hendrickson and Burgess (1989)	2.8	2.2
Robson moraines, British Columbia	Boreal Forest	-119	53	ARA	Blundon and Dale (1990)	1.1	1.2
Umea, Sweden	Boreal Forest	19.5	64	ARA	Huss-Danell (1976)	1	1.5
Coweeta Basin,	Temperate Forest	-83	35	N accumulation	Boring and Swank (1984)	48	19.5
Hoh River, Washington, US	Temperate Forest	-123.5	48	ARA	Luken and Fonda (1983)	40	13
Tom Swamp, Massachusetts, US	Temperate Forest	-75	42.5	unspecified	Schwinzer (1983)	35	25.7
Big Creek Basin, Melbourne, Australia	Temperate Forest	145.5	38	ARA	Adams and Attiwill (1984)	24	23.2
Jebo Creek, Utah, US	Temperate Forest	-112	42	¹⁵ N	Skujins et al. (1987)	10.2	12.5
Karri Forest, south-western Australia	Temperate Forest	116	-34.5	ARA	Grove and Malajczuk (1992)	7.93	8.5
Woodhill Forest, New Zealand	Temperate Forest	174.5	-37	N accumulation	Baker et al. (1986)	80	23.5
Gainesville, Florida, US	Temperate Forest	-82	30	N accumulation	Permar and Fisher (1983)	10.6	12.8
Fox park, Wyoming, US	Temperate Forest	-106	41	ARA	Fahey et al. (1985)	13	12.5
Mount Robson, Canada	Temperate Forest	-119	53.1	ARA	Blurdon and Dale (1990)	1.65	3.2

Dwellingup, South-western Austrilia	Temperate Forest	116	33	ARA	O'Connel and Grove (1987)	2.5	3.1
Adair, Oregon, US	Temperate Forest	-123	44.6	ARA	Heath et al (1988)	0.74	2.4
Priest River Experimental Forestry, Idaho, US	Temperate Forest	-116	48	ARA	Harvey et al (1989)	0.1	1.7
Arapaho Prarie, Nebraska, US	Grassland	-100	42	unspecified	Kaputsa and DuBois (1987)	0.2	0.7
Lynx Prairie Preserve, Ohio, US	Grassland	-83.5	39	ARA	DuBois and Kaputsa(1983)	8.2	1.9
Konza Prarie Research Natural Area, Kansas, US	Grassland	-96	39.5	nitrogenase activity	Eisele et al (1989)	21	3.3
Buso, Papua New Guinea	Tropical Forest	147	-7.5	ARA	Goosem and Lamb (1986)	0.5	5.2
Reserve Ducke, Manaus, Brazil	Tropical Forest	-59	-3	ARA	Sylvester-Bradley et al. (1980)	2.45	3.5
Sinharaja Man and Biosphere reserve	Tropical Forest	80.5	6.5	ARA	Maheswaran and Gunatilleke (1990)	8	8.5
Amazon Territory of Venezuela	Tropical Forest	-67	2	ARA	Jordan et al (1983)	32	20.3
Kilauea, Hawaii, US	Tropical Forest	-155	19	ARA	Vitousek (1994)	2.8	18.5
Volcano La Soufriere, Guadeloupe	Tropical Forest	-61.5	16	ARA	Sheridan (1991)	4.02	7.2
Hawaii Volcanoes National Park, US	Tropical Forest	-155	19.5	ARA	Ley and D'Antonio (1998)	4.9	9.3
Santa Ynez Mountain, California, US	Mediterranean Shrubland	-120	34.5	ARA	Schlesinger et al.(1982)	1	2.4
San Bernardino Mountains, California, US	Mediterranean Shrubland	-116.5	34	ARA	Lepper and Fleschner (1977)	6.9	3.7
Harpers Well, California, US	Xeric Shrubland	-116	33.5	N accumulation	Rundel et al.(1982)	30	18.5
Sonoran Desert, Arizona, US	Xeric Shrubland	-112.5	33	cation accumulation	Jarrell and Virginia (1990)	40	23.5

Table 3. Sites used for model validation of N₂O emissions

	Ecosystem			T	Precipitation	Length of	N₂O Fluxes	
Site name	Type	Lon.	Lat.	(°C)	(mm)	experiment (day)	kg N ha⁻¹ yr⁻¹	Reference
1 Linden, Giessen,	Temperate							Müller and Shelock
Germany	forest	8.5	50.5	1–20	0–14	400	0.292	(2004)
2 Horquetas, Costa Rica	Savanna	-85	10	25.8	3962	23–30	4.3	Veldkamp et al. (1998)
3 Yurimaguas, Peru	Rainforest	-76	-6	26	2200	365	0.8	Palm et al. (2002)
4 Central Scotland	Grassland	-4.5	56.5	12.6	129.3	7	0.02–0.09	Skiba et al. (1998)
5 Wagga Wagga	Grassland	147.5	-35.5	3–31	570	90	0.35	Galbally et al. (2009)

Table 43. Model estimated biological nitrogen fixation in global natural terrestrial ecosystems

Ecosystem	Average coverage of N fixing plants	Coverage range	Reference	N Fixation Rate (kg N ha⁻¹ yr⁻¹)	Total_Min (Tg N yr⁻¹)	Total_Max (Tg N yr⁻¹)	Total_Avg (Tg N yr⁻¹)	Area (10⁸ ha)
wet tundra	9%	3%~15%	May and Webber (1982)	3.2	0.51	2.55	1.54	5.37
alpine tundra & wet tundra	9%	3%~15%	May and Webber (1982)	3.2	0.51	2.55	1.54	5.36
boreal forest	9%	4%~18%	Alexander and Billington (1986); weber and Van Cleve (1981)	2.1	2.01	9.06	4.53	19.3
temperate coniferous forest	5%	1%~10%	Cleveland et al (1999)	12.7	0.71	7.15	3.5	5.51
temperate deciduous forest	5%	1%~10%	Cleveland et al (1999)	12.7	0.76	7.65	3.75	5.89
temperate evergreen forest	5%	1%~10%	Cleveland et al (1999)	12.7	0.43	4.34	2.13	3.35
grassland	15%	5%~25%	Woodmansee et al (1981); Robertson and Rosswall (1986)	1.9	0.61	3.1	1.86	8.4
tropical forest	15%	5%~25%	Cleveland et al (2001)	18.2	10.8	54	32.6	17.8
xeric shrubland	15%	10%~20%	Johnson and Mayeux (1990)	5.7	2.92	14.6	8.35	14.8
Mediterranean shrubland	15%	10%~20%	Johnson and Mayeux (1990)	2.7	0.13	0.66	0.4	1.47
savanna	15%	5%~25%	Stewart et al (1978); Bate and Gunton (1982)	1.9	0.45	2.23	1.34	7.05
Total					19.84	107.89	61.54	94.3

Table 54. Model parameters for various natural terrestrial ecosystems

	N_pot (g N fixed day ⁻¹)	t_optL (°C)	t_optH (°C)	W_upH (J kg ⁻¹)	fNup	Kc (g C m ⁻²)
1 wet tundra	0.028	10	25	0.8	65	0.002
2 alpine tundra & wet tundra	0.028	10	25	0.8	65	0.002
3 boreal forest	0.032	12	25	0.8	70	0.008
4 temperate coniferous forest	0.55	16	35	0.6	80	0.01
5 temperate deciduous forest	0.55	18	35	0.6	80	0.01
6 temperate evergreen forest	0.55	18	35	0.6	80	0.01
7 grassland	0.05	18	35	0.5	60	0.012
8 tropical forest	0.8	20	35	0.8	100	0.005
9 xeric shrubland	0.7	15	35	0.4	65	0.016
10 Mediterranean shrubland	0.08	19	35	0.5	65	0.016
11 savanna	0.05	20	35	0.5	60	0.012

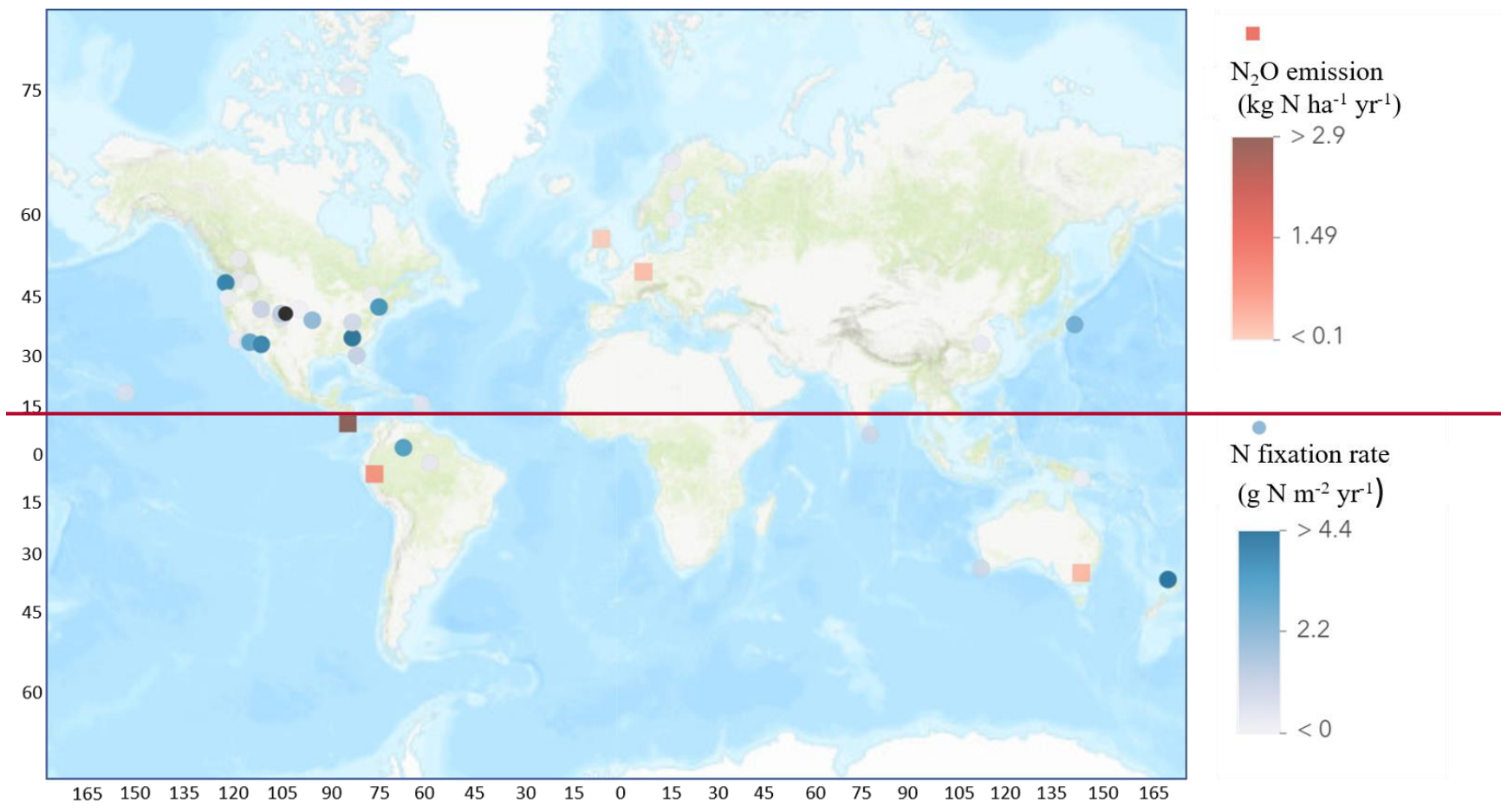
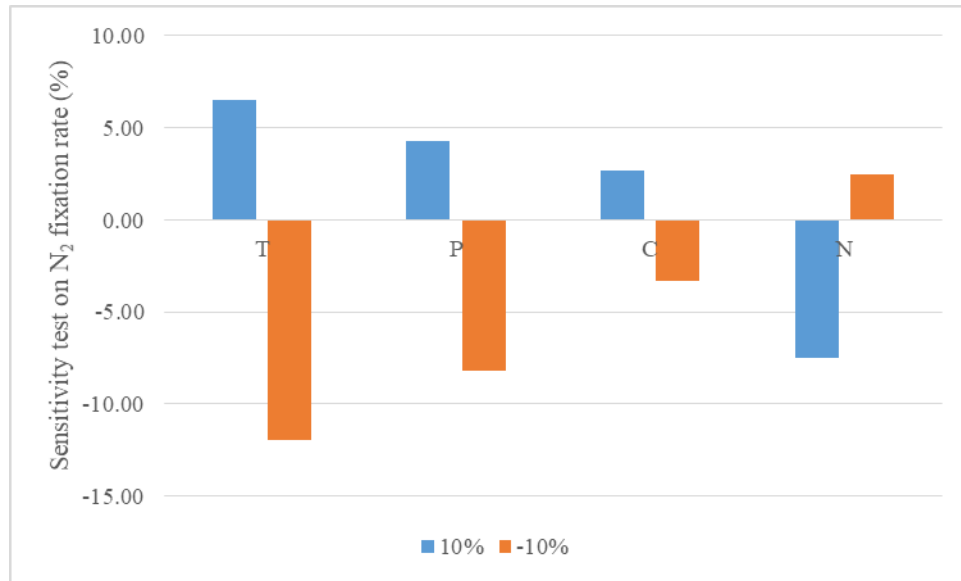


Figure 1. Sites used with observation data of N₂ fixation rate (Blue) and N₂O emissions (Red) for model parameterization

(a)



(b)

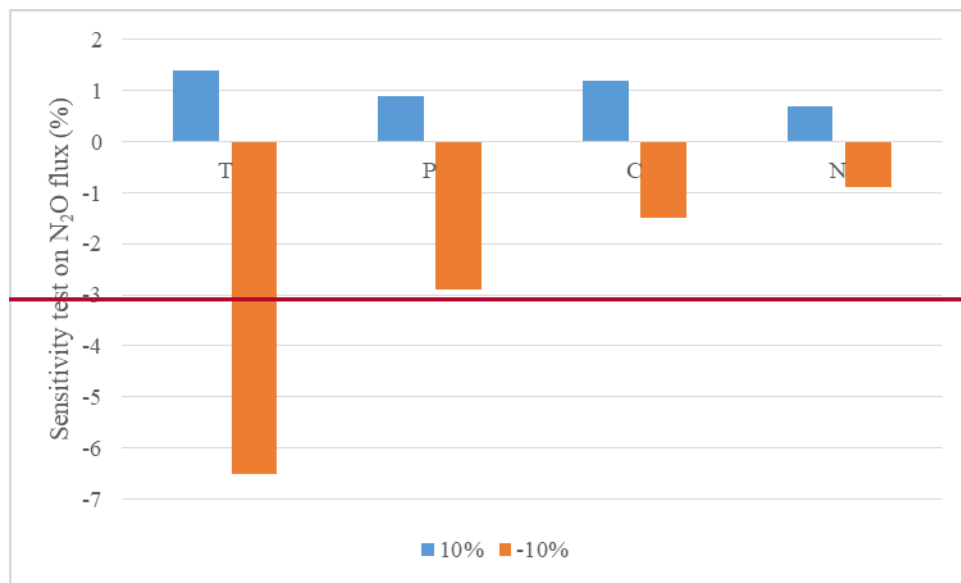
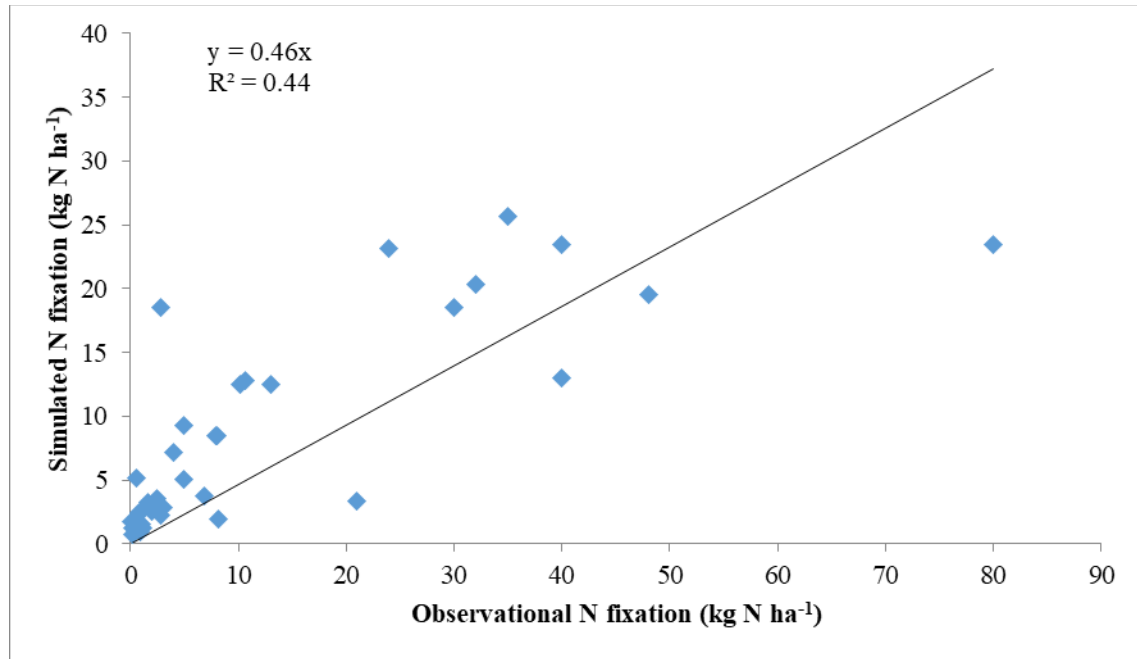
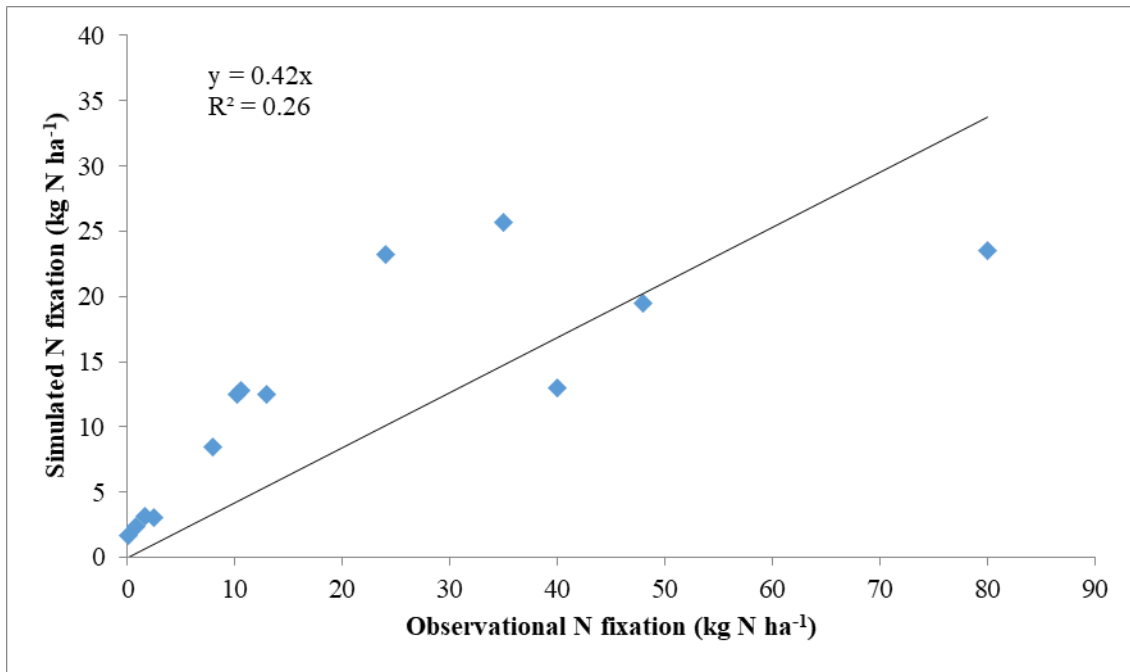


Figure 21. Model sensitivity of N fixation and N₂O emissions in natural terrestrial ecosystems to changing model input data: Increasing or decreasing each variable by 10% for air temperature (T), precipitation (P), soil carbon content (C), soil nitrogen content (N) for (a) N fixation rate, and (b) N₂O emissions

(a)



(b)



(c)

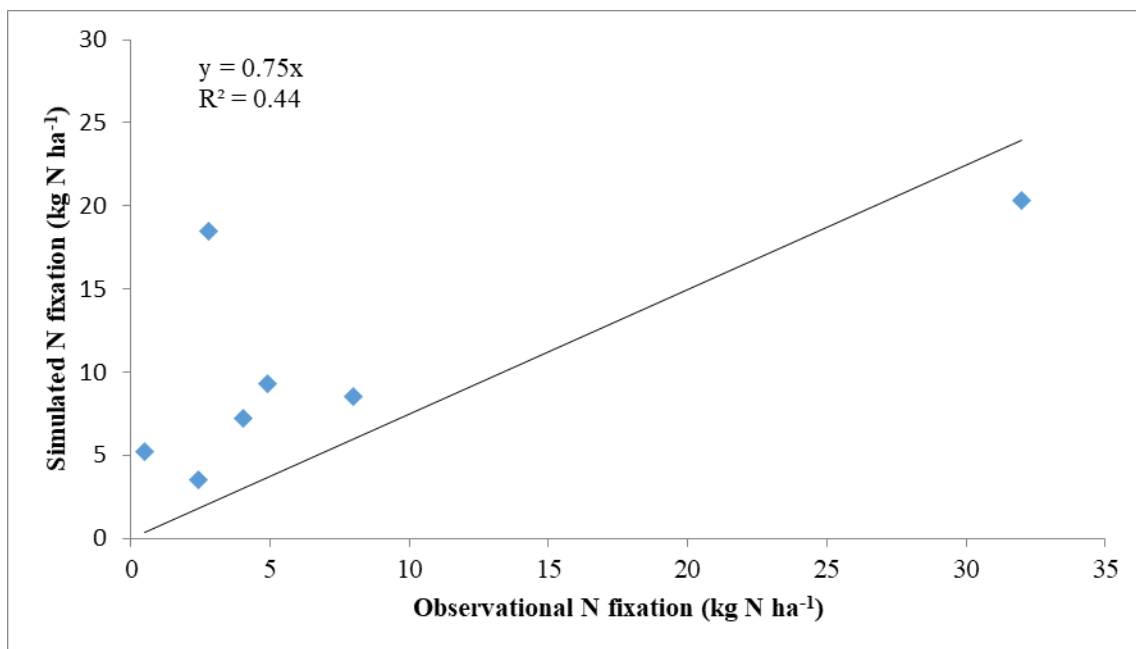
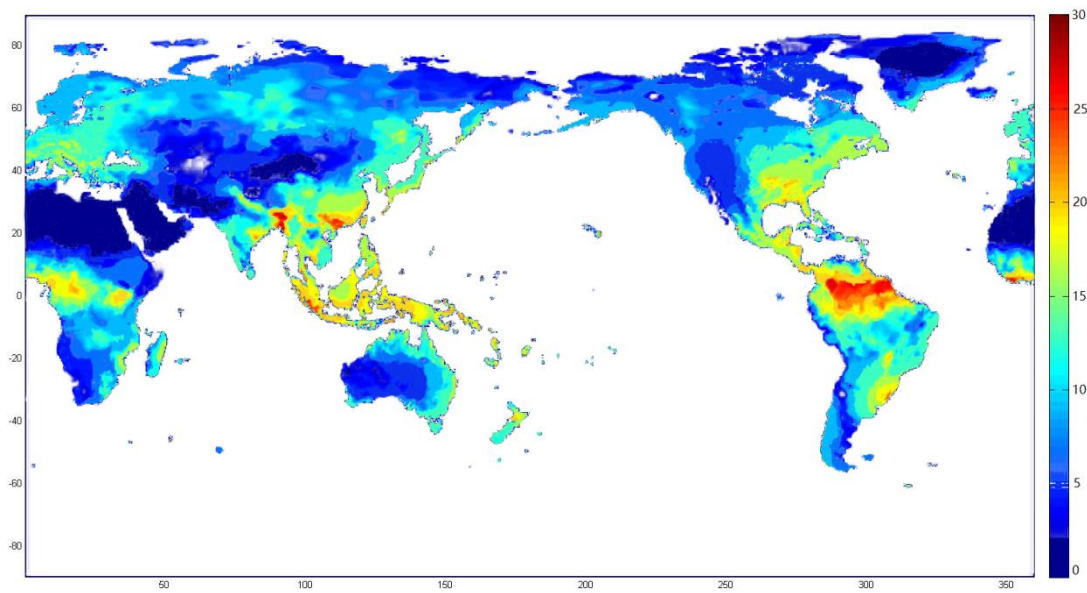


Figure 13. Comparison between Modeled and Observed Nitrogen Fixation Rate at site level: (a) All sites, (b) Temperate Forest, (c) Tropical Forest (data listed in Table 2). Y is simulated N fixation while X represents the observational N fixation.

(a)



(b)

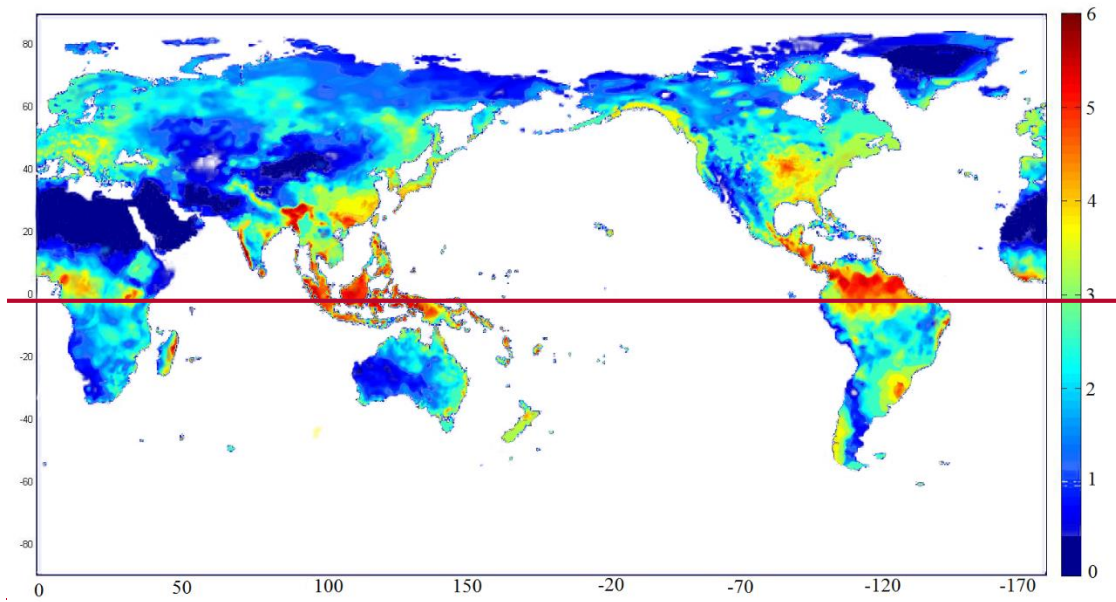
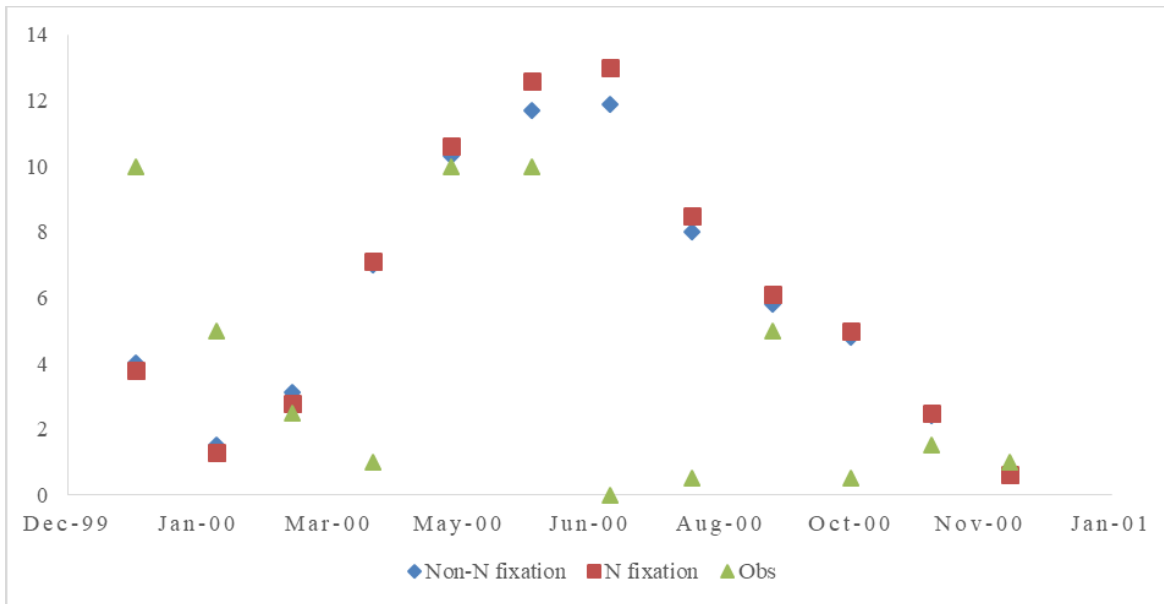
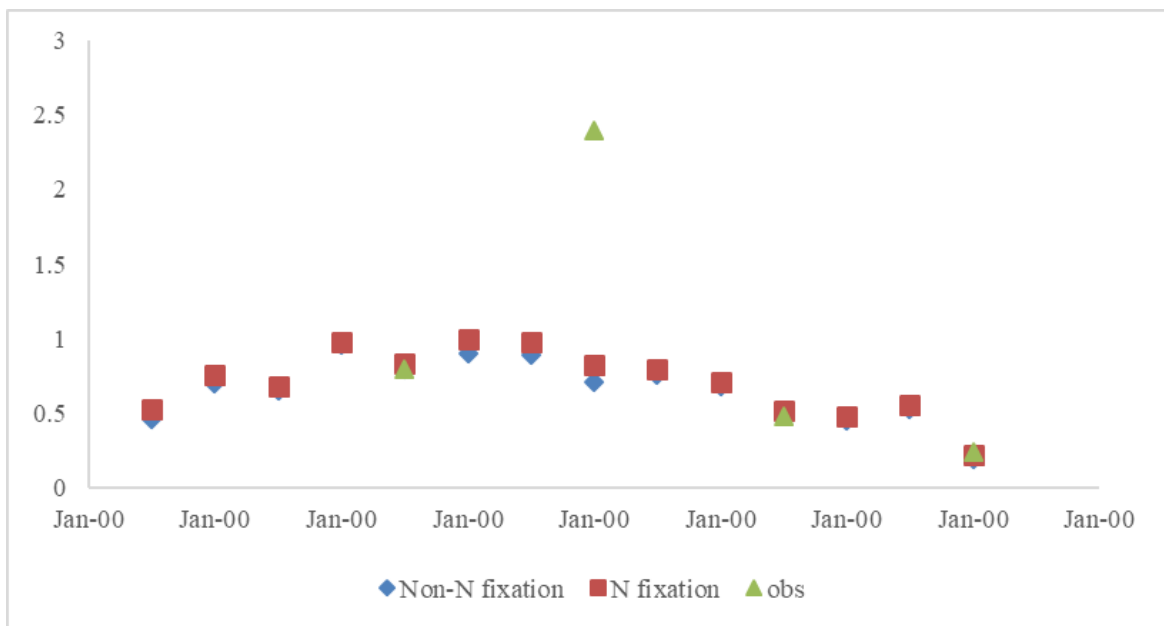


Figure 4. Simulated spatial distribution of (a) BNF rates ($\text{kg N ha}^{-1} \text{ yr}^{-1}$); (b) N_2O emissions ($\text{kg N ha}^{-1} \text{ yr}^{-1}$) in natural terrestrial ecosystems during 1990-2000 by considering the BNF effects.

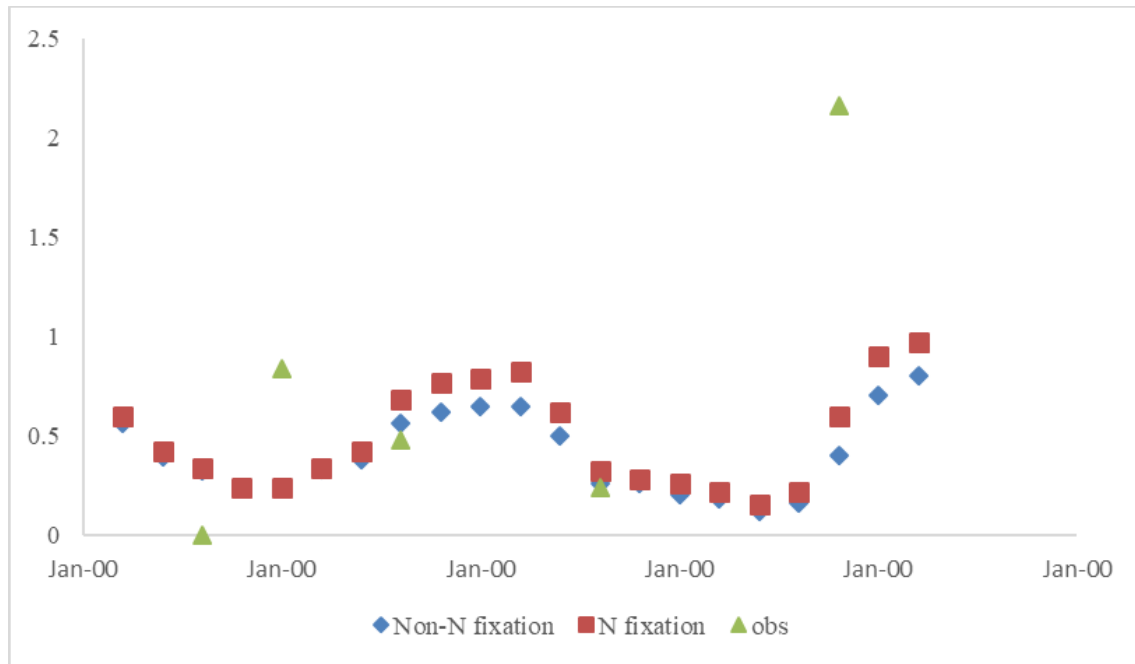
(a)



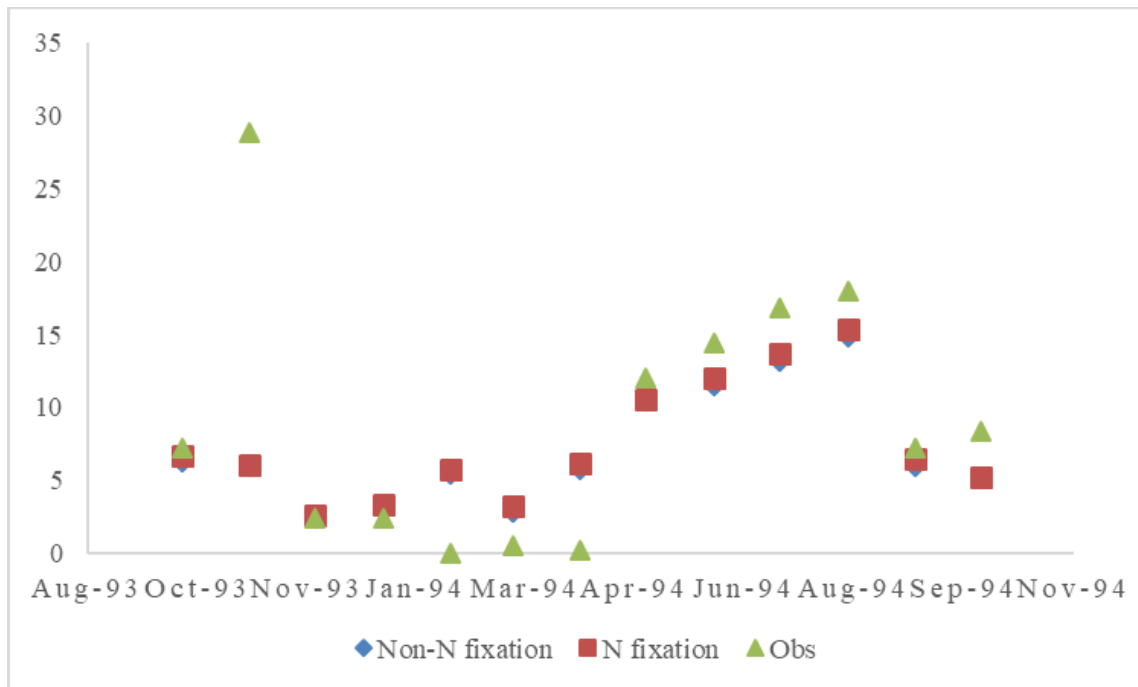
(b)



(c)



(d)



(e)

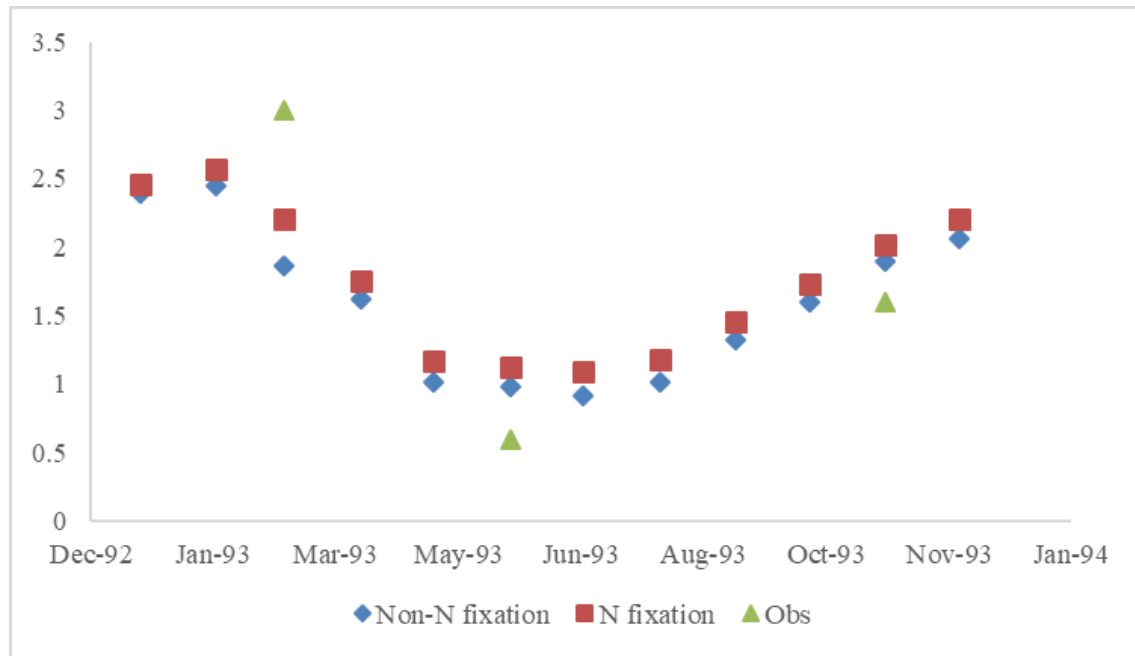


Figure 5. Comparison between simulated and observed N₂O emissions (g N m⁻² day⁻¹) with two versions of the model (Red indicates the simulations considering BNF and blue indicates the simulations without considering BNF effects on N₂O emissions) at sites: (a) Temperate Forest (50.5°N, 8.5°E); (b) Tropical Forest (10°S, 63°W); (c) Savanna (10°N, 85°W); (d) Tropical Forest (6°S, 76°W).