# nature portfolio

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## **Reporting Summary**

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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For	all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Confirmed
	$\square$ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
	The statistical test(s) used AND whether they are one- or two-sided  Only common tests should be described solely by name; describe more complex techniques in the Methods section.
	A description of all covariates tested
	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

Policy information about <u>availability of computer code</u>

Data collection

Provide a description of all commercial, open source and custom code used to collect the data in this study, specifying the version used OR state that no software was used.

Data analysis

JMP16.2.0, R version 4.2.3, Trimmomatic (ver. 0.39), BWA (ver.0.7.17), samtools (ver. 1.12), Genome analysis tool kit (GATK ver. 4.0.11.0), SnpEff (ver.4.3), Manta (ver.1.6.0), SURVIVOR (ver.1.0.7), Beftools (ver.1.13)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio <u>guidelines for submitting code & software</u> for further information.

#### Data

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support the findings of this study are available from the corresponding author, IMAIZUMI-ANRAKU.H, upon reasonable request.

### Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one I	pelow that is the best fit for your research.	f you are not sure, read the appropriate sections before making your selection.
□ Life sciences	Behavioural & social sciences	Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <a href="mailto:nature.com/documents/nr-reporting-summary-flat.pdf">nature.com/documents/nr-reporting-summary-flat.pdf</a>

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

Sample size was not determined based on statistical analysis.

We judged that the number of samples shown in the figures and tables would be sufficient for statistical analysis, because we had already grasped the trend of the data shown in the figures from multiple experiments under the similar conditions. The information for each figure is provided in Replication.

Data exclusions

Based on the outlier analysis in JMP, outliers were determined and removed from the data.

Replication

Figures 1, 4, 5, and SplFigure 4 show reproducibility in different experiments under the same conditions. For SplFigure 2, the negative correlation between the root nodule occupancy rate and N2O emission rate was not clear in different experiments under the same conditions. For Figure 3, the statistical power of samples tested is greater than 0.9, so there is no problem in setting the number of samples. For Figure 6, although plots were not randomized due to field management constraints, six individuals from each plot were examined for nodule occupancy and four for N2O flux to verify reproducibility.

Randomization

The experiments were carried out by multiple people, and the assignment of samples to be handled at each time was random.

Blinding

Blinding was not performed. The data was shared among multiple people, and after confirming that the analysis results were consistent among them, the analysis results were unified and presented as data.

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experime	ntal systems Methods			
n/a Involved in the study	n/a Involved in the study			
Antibodies	ChIP-seq			
Eukaryotic cell lines	Flow cytometry			
Palaeontology and a	rchaeology MRI-based neuroimaging			
Animals and other o	rganisms			
Clinical data				
Dual use research of	· concern			
Plants				
Dual use research	of concern			
Policy information about <u>du</u>	ual use research of concern			
Hazards				
Could the accidental, deli in the manuscript, pose a	berate or reckless misuse of agents or technologies generated in the work, or the application of information presented			
No Yes	tilleat to.			
Public health				
National security				
Crops and/or livest	OCK			
Ecosystems				
Any other significa	nt area			
Experiments of concer	'n			
Does the work involve an	y of these experiments of concern:			
No Yes				
Demonstrate how	to render a vaccine ineffective			
Confer resistance t	to therapeutically useful antibiotics or antiviral agents			
Enhance the virule	nce of a pathogen or render a nonpathogen virulent			
Increase transmissi	ibility of a pathogen			
Alter the host rang	Alter the host range of a pathogen			
Enable evasion of diagnostic/detection modalities				
Enable the weapor	Enable the weaponization of a biological agent or toxin			
Any other potentia	lly harmful combination of experiments and agents			
Plants				
Seed stocks	NARO genebank, JP28493(Bonminori), JP27584(Karasu-mame), JP28320(Akuden shirazu)			
Novel plant genotypes	No applicable.			
Authentication	No applicable.			