Electricity-powered artificial root nodule

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Root nodules are agricultural-important symbiotic plant-microbe composites in which microorganisms receive energy from plants and reduce dinitrogen (N₂) into fertilizers. Mimicking root nodules using artificial devices can enable renewable energy-driven fertilizer production. This task is challenging due to the necessity of a microscopic dioxygen (O₂) concentration gradient, which reconciles anaerobic N₂ fixation with O₂-rich atmosphere. Here we report our designed electricity-powered biological|inorganic hybrid system that possesses the function of root nodules. We construct silicon-based microwire array electrodes and replicate the O₂ gradient of root nodules in the array. The wire array compatibly accommodates N₂-fixing symbiotic bacteria, which receive energy and reducing equivalents from inorganic catalysts on microwires, and fix N₂ in the air into biomass and free ammonia. A N₂ reduction rate up to 6.5 mg N₂ per gram dry biomass per hour is observed in the device, about two orders of magnitude higher than the natural counterparts.

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A major component of global dinitrogen (N₂) fixation occurs in root nodules, the symbiotic plant–microbe composites in which microorganisms receive energy from plants and reduce N₂ into fertilizers. The significance of biological root nodules in agriculture inspires us to consider a man-made mimic, which houses diazotrophs and fixes N₂ in air with renewable energy such as solar electricity. Biological–inorganic hybrid systems are reported to use sustainable energy, such as light and renewable electricity to drive biochemical reactions with high throughput and energy efficiency. We recently reported a biocompatible system that utilizes electricity to power the N₂ fixation in Xanthobacter autotrophicus, a non-symbiotic root-associative diazotroph. In this system, water is split into O₂ and H₂ with small thermodynamic driving forces. The generated H₂ as a reducing equivalent is selectively consumed by the hydrogenases in autotrophic microorganisms and powers the biochemical reduction of carbon dioxide (CO₂) and N₂. However, this hybrid system operates at a microaerobic condition of 2% O₂ other than ambient air, which limits its potential for practical applications. In root nodules, the anaerobic nature of N₂-fixing nitrogenases is incompatible with the aerobic metabolism in plant cells. In order to accommodate this incompatibility for a successful N₂ reduction in air, the O₂ environment in root nodules quickly changes from aerobic at the apex to hypoxic towards the base across tens or hundreds of micrometers. Such a steep O₂ gradient in living organisms has been challenging to mimic with a man-made device. Designs of hydrogel and microfluidic systems have been employed to create steady-state concentration profiles, yet these delicate setups require a continuous gas purging or supply of O₂ scavengers, which are not readily tunable or attainable with long-term stability. While biofilms hosting diazotrophs have been recently reported to exhibit N₂ fixation in air, their complex and less-well-defined nature renders certain challenges.

Here we report our strategy that uses microwire array electrode to create a controllable O₂ gradient for the construction of an electricity-driven artificial root nodule (Fig. 1b). The wire array compatibly accommodates N₂-fixing symbiotic bacteria within and provides energy and reducing equivalents to the bacteria. Resembling the natural root nodules, the generated local hypoxic environment within the microwire array enables the biological N₂-fixation in air. The reported biological–inorganic hybrid system can potentially supplement non-legume crops with electricity-generated fertilizers and reduce the reliance on synthetic alternatives.

Results

Design principle and numerical simulations. In our design, microwire array electrode provides a suitable platform of delivering reducing equivalents to biocatalysts, as well as establishing a controllable O₂ environment for biological N₂ fixation in air (Fig. 1b). Under a cathodic electrochemical potential, the large surface area of wire array electrode offers fast transfer of reducing equivalents to the microbes through either a direct or a mediated pathway (Fig. 1b). Moreover, in air, the electrochemical...

Fig. 1 Scheme and the simulated O₂ gradient of electricity-driven artificial root nodule. Scheme of N₂ fixation in the natural a and the proposed artificial root nodule b. c Morphology of designed microwire array and the cross-sectional display of simulated O₂ concentration ([O₂]) profile. Length (l), 50 μm; diameter (d), 4 μm; periodicity (p), 15 μm; diffusion layer thickness (dₘ), 20 μm. d Simulated [O₂] against the distance from the base of electrode (z) for planar electrode and microwire arrays of different p values (l = 50 μm, d = 4 μm). The hypoxic (pink) and aerobic (blue) domains for wire array of p = 15 μm are suggested. e Scanning electron microscopy (SEM) image of the prepared Si wire array with a morphology shown in c. ITO indium-tin oxide. Scale bar, 15 μm. Electrochemical potential (Eᵯₒᵢᵣ) = 0.5 V vs. reversible hydrogen electrode (RHE) in c and d.
reduction of O$_2$ on electrode’s surface consumes O$_2$ and creates a local O$_2$ gradient in the solution. This effect is much more pronounced for porous and large-surface-area electrodes in general\textsuperscript{24-27}, effectively creating an O$_2$-free domain within the wire array suitable for anaerobic N$_2$ fixation (Fig. 1b). Our previous work demonstrated a O$_2$-free domain within a nanowire array electrode\textsuperscript{28-30}, which allows anaerobic microbial reduction of CO$_2$ in air\textsuperscript{23}. Here we apply the same design principle for an artificial root nodule. Numerical simulations based on a micro-kinetic model were applied to design a suitable wire array geometry for biological N$_2$ fixation (see "Methods" section).

When the wire electrode was at an electrochemical potential ($E_{\text{appl}}$) cathodic enough to reduce O$_2$ ($E_{\text{appl}} = 0.5$ V vs. reversible hydrogen electrode, RHE) at pH $= 7.0$ (same below), the O$_2$ concentration near the electrode ([O$_2$]) quickly decreases towards the base of wire array (Fig. 1c), establishing an O$_2$ gradient more significant than the case when a planar electrode is employed (Fig. 1d)\textsuperscript{19}, suitable for biological N$_2$ fixation (see Supplementary Note 2)\textsuperscript{5,12}.

**Fabrication and characterization of wire array electrodes.** The microwire array device, designed based on numerical simulation (Fig. 1c, d), was constructed for electricity-driven N$_2$ fixation in air. A five-step fabrication process (see "Methods" section) led to a core–shell microwire array, which contains a silicon (Si) microwire core, a SiO$_2$ insulating coating, a layer of indium-tin oxide (ITO), and Pt particles deposited via sputtering (Fig. 1b)\textsuperscript{28}. The electrically conductive ITO layer ensures a uniform distribution of $E_{\text{appl}}$ on wires’ surface; the Pt particles not only transfer reducing equivalents to diazotrophs for N$_2$ fixation but also electrochemically consume O$_2$ for a controlled O$_2$ gradient\textsuperscript{29,30}. The morphology and composition of the wire array were characterized and confirmed by scanning electron microscopy (SEM) equipped with energy-dispersive X-ray spectroscopy (EDS) (Fig. 1c and Supplementary Fig. 2). Linear scan voltammograms (LSVs) of the array electrode in an all-inorganic medium for diazotrophs displayed an onset potential of O$_2$ reduction at 0.8 V vs. RHE and a noticeable generation of H$_2$ as the energy source\textsuperscript{5,33}, were inoculated separately into microwire array electrodes shown in Fig. 1c. In an air-equilibrated liquid medium deprived of any nitrogen and organic carbon sources (see "Methods" section), an electrochemical potential ($E_{\text{appl}} = -0.15$ V vs. RHE) was supplied to establish the proposed O$_2$ gradient and deliver reducing equivalents, possibly H$_2$, for biological N$_2$ fixation (Fig. 3a). In 120 h after inoculation, confocal fluorescence microscopy images (see "Methods" section) indicate significant increase of microbial population and biomass for both $B$. japonicum and $X$. autotrophicus strains (Fig. 3b, c and Supplementary Fig. 7). As the only possible source of nitrogen and carbon in the biomass is the N$_2$ in the air and CO$_2$/bicarbonate in the medium, such a biomass increase suggests biological N$_2$ and CO$_2$ immobilization. In contrast, when we inoculated a $B$. japonicum variant unable to fix N$_2$ with its disrupted nifH gene ($B$. japonicum-H1)\textsuperscript{34,35}, negligible biomass accumulation was observed under the same condition (Supplementary Fig. 8). This indicates that the observed biomass accumulation is concurrent with the expression of nifH protein essential to N$_2$ reduction. Last, when there was not enough driving force ($E_{\text{appl}}$) from electricity to drive N$_2$ fixation, no net accumulation of biomass was observed (Supplementary Fig. 9). It supports our claim that electricity is the sole energy source of N$_2$ fixation.

After 120 h since inoculation, quantitative assays (see "Methods" section) show that the changes of microbial cell counts (ACC) within the wire array were $4.0 \pm 1.5 \times 10^9$ and $4.2 \pm 1.6 \times 10^9$ cells mL$^{-1}$ for $X$. autotrophicus and $B$. japonicum strains, respectively (entries 1 and 2 in Fig. 3d; $n = 5$, $\pm$ denotes standard deviation, same below). The final microbial cell counts in the device are 19 and 2.5 times of the values right after the inoculation (2.2 $\pm 1.2 \times 10^8$ and $2.7 \pm 1.8 \times 10^9$ cells mL$^{-1}$) for $B$. japonicum and $X$. autotrophicus, respectively. This highlights that the developed system is biocompatible even for symbiotic microorganisms that might demand additional stimuli for N$_2$ fixation (Supplementary Note 3)\textsuperscript{4,36}. In correspondence to the change of cell counts, the changes of total nitrogen content (ATN) retained in biomass in the
device, an indicator of biological N2 fixation, were 5.7 ± 2.3 × 10^2 and 1.9 ± 0.7 × 10^2 mg L^-1 for X. autotrophicus and B. japonicum strains, respectively; the changes of total organic carbon (ΔTOC), another indicator of microbial biomass accumulation, were 2.5 ± 1.0 × 10^3 and 6.9 ± 2.5 × 10^2 mg L^-1 for X. autotrophicus and B. japonicum strains, respectively. Moreover, we observed the accumulation of free ammonia in the ef fluent out of the electricity-driven N2 fixation device. When 20 mL of medium was circulated throughout the device during a 120-h application of electricity, the nitrogen content in terms of ammonia in the liquid ef fluent was measured to increase by 0.017 ± 0.003 mg L^-1 for B. japonicum; no detectable free ammonia was observed for X. autotrophicus strain under the same experimental condition, consistent with our prior report with X. autotrophicus strains in the absence of any external inhibitor12 (see “Methods” section). This implies that under a flow condition part of the fixed ammonia is capable to passively diffuse extracellularly, resembling the scenario in the symbiotic system within a root nodule37. As far as we know, microbial-based free ammonia production from electricity has not yet been reported except our prior work that

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**Fig. 2 Experimental validation of microscopic O2 gradient in microwire array.** a Simplified Jablonski diagram of Ru(phen)$_3^{2+}$ including the absorption (A), intersystem crossing (ISC), phosphorescence (P), and the quenching (Q) of its triplet excited states by O2. $l_{em}$ and $\tau$ denote the intensity and lifetime of phosphorescence emission, respectively. b Electrochemical setup under a confocal microscope that in situ maps O2 gradient. WE denotes microwire array working electrode and CE denotes counter electrode. The setup is mounted on an inverted microscope so the wire array is facing down during observation (as shown in Supplementary Fig. 4). c, d Cross-sectional $l_{em}$ profiles in wire array in the absence and presence of $E_{appl}$, respectively. Dashed lines depict wire morphology. Scale bars, 15 μm. e The temporal response of relative intensities $l_{em}$ ($I_t/I_0$) at different z values within wire array. $E_{appl}$ was switched on and off at $t$ = 15 and 35 s, respectively (n = 3). f Simulated (lines) and experimentally determined (points, n = 3) [O2] profiles at different electrode geometries. Unless specified, all results, as well as the suggested hypoxic (pink) and aerobic (blue) areas, refer to the geometry shown in Fig. 1c under $E_{appl}$ = 0.5 V vs. RHE. Error bars = standard deviation (n = 3).
utilized inhibitor to purposely prevent microbes from up-taking ammonia\textsuperscript{12}, a non-ideal practice for long-term operation. Overall, the electricity-driven artificial root nodule is capable of fixing N\textsubscript{2} in air at a rate of 6.5 and 1.1 mg N\textsubscript{2}/g dry biomass per hour for \textit{B. japonicum} and \textit{X. autotrophicus} strains, respectively (see “Methods” section). Such rates of N\textsubscript{2} fixation in our system are about two orders of magnitudes higher than their natural counterparts in the symbiotic root nodules with higher energy efficiencies (see “Methods” section, Supplementary Table 1 and Supplementary Note 4)\textsuperscript{1,38}.

Fig. 3 Electricity-driven N\textsubscript{2} fixation in air. a Schematic illustration of the functional device. b, c Fluorescent images of \textit{B. japonicum}, indicative of biomass accumulation, before \textbf{b} and after \textbf{c} 120-h operation in microwire array. \textit{E}_{\text{appl}} = -0.15 V vs. RHE, \(p = 15 \mu\text{m}\), images taken at \(z = 20 \mu\text{m}\). d Changes of cell count (\(\Delta\text{CC}\)), as well as the retained total nitrogen (\(\Delta\text{TN}\)) and total organic carbon (\(\Delta\text{TOC}\)) in biomass in the device, calculated as the differences between values before and after 120-h operation with the provision of electricity (\(n = 5\)). \(\infty\), planar electrode; \textit{Xa} \textit{X. autotrophicus}; \textit{Bj} \textit{B. japonicum}, \textit{Bj-H1} \textit{B. japonicum-H1}, a \textit{B. japonicum} variant with disrupted \textit{nifH} gene. e Integrated fluorescent intensities of \textit{X. autotrophicus}, indicative of biomass accumulation, at different \(z\) positions for different electrode morphologies. The hypoxic (pink) and aerobic (blue) domains for wire array of \(p = 15 \mu\text{m}\) are suggested (\(n = 5\)). f, g Series of x-y cross-sectional fluorescent images at different \(z\) planes for \textit{X. autotrophicus} population within wire arrays of \(p = 15 \mu\text{m}\) \textbf{f} and \(p = 30 \mu\text{m}\) \textbf{g}. Pseudo-colored in green. Scale bars, 30 \(\mu\text{m}\). The setup for imaging is mounted on an inverted microscope so the wire array is facing down during observation (as shown in Supplementary Fig. 4). For panels \textbf{d} and \textbf{e}, dot-plot of individual measurements is overlaid with the bar graph. Error bars = standard deviation (\(n = 5\)).
The established O2 gradient within the wire array is crucial to the N2 fixation. The established O2 gradient within the wire array is crucial to the functionality of biological N2 fixation in air. Wire array in the absence of deposited Pt particles did not lead to observable N2 fixation (Supplementary Fig. 9). It suggests that Pt particles are effective towards oxygen reduction, enable the establishment of O2 gradient, and deliver reducing equivalents to the microbes via reducing protons or possibly other direct routes of charge transfer (Supplementary Note 5). Moreover, the microbial distribution at different depths of the wire array (Fig. 3e), characterized by confocal microscopy, suggests a strong correlation between the O2 gradient and the microbial population density. When planar electrode or wire array of \( p = 30 \) µm were inoculated with diazotrophs (entries 4 and 5 in Fig. 3d, respectively), minimal N2 fixation was observed. While the wire array of \( p = 15 \) µm exhibits high microbial density until the very top (\( z > 40 \) µm in Fig. 3e), which is in accordance with the O2 gradient measured under the same \( E_{\text{appl}} \) for nitrogen fixation experiment (Supplementary Note 6 and Supplementary Fig. 10), planar substrate or wire array of larger \( p \) value (\( p = 30 \) µm) did not display significant microbial accumulation. Such a difference is also visible in Fig. 3f (\( p = 15 \) µm) and Fig. 3g (\( p = 30 \) µm), which highlight the active diazotrophs at different depths of wire arrays. These results illustrate that microwire array not only delivers reducing equivalents for N2 fixation, but also creates the hypoxic domains in air as the root nodules do.

**Discussion**

Designing wire array electrodes provide a unique and viable approach to program O2 gradient at microscopic length scale with high fidelity. Our reported proof-of-concept demonstrates that the established O2 gradient enabled by micro-structured electrodes allows the production of nitrogen fertilizer by O2-sensitive microbes in air using sustainable electricity (Supplementary Fig. 11), replacing the carbohydrates from natural photosynthesis as the driving force for biological N2 fixation. The observed generation of free ammonia from symbiotic microorganisms driven by electricity, differs from prior result of planktonic species, which highlights the unique biocatalytic utilities of symbiotic diazotrophs in the future. Our demonstration also offers the design principle of porous electrodes (Supplementary Note 7) for future N2-fixing variants that only contains biocompatible earth-abundant materials (Supplementary Note 8), provides lower cost and better scalability with ingenious engineering design, and employs genetically modified microbes with higher intrinsic rates of N2 fixation. In the long run, the concept of biological/inorganic hybrid system will potentially supplement non-legume crops with electricity-generated fertilizers and reduce the reliance on synthetic alternatives.

**Methods**

**Chemicals and materials.** The chemicals, unless otherwise specified, were purchased from Sigma-Aldrich and used as received. The ITO-coated glass slides were obtained from SPI supplies. Silicon wafers were purchased from University Wafer. Silver paint was purchased from Ted Pella. Chemical-resistant tapes were purchased from McMaster-Carr. Plastic tubing and fittings were obtained from IDEX Health & Science. Polystyrene plates were purchased from Evergreen Scale Models. A-B epoxy adhesives were obtained from Amazon. Health & Science. Polystyrene plates were purchased from Evergreen Scale Models.

**Preparation procedure of microwire array electrode.** Si wire array was prepared using photolithography followed by reactive ion etching\(^{32}\), 500 µm-thick, 4" Si wafer (p-type, boron-doped, (100) facet-oriented, 1–10.0 cm\(^{-1}\)), University wafer) was patterned by a contact aligner (Carl Suss MA6) with photoresist (Micro-Chemicals AZ5214E), and was then developed with MicroChemicals AZ 400K. The microwire array morphology was created by a subsequent reactive ion etching (Unaxis Versaline, FDSE III). A layer of protective silicon dioxide layer on the surface of wire array was created by thermal annealing in air at 1050 °C for 9h in a tube furnace (Lindberg/Blue M, Fisher Scientific). A conformal layer of ITO with 200-nm thickness was coated via reactive sputtering using ULVAC RF sputtering system. Pt particles of 2 nm thickness was deposited on the wire’s surface with a Anatech Hummer 6.2 sputtering system. The morphology of prepared wire array was characterized by a SEM (JEOL, JSM-6701F) equipped with energy dispersive X-ray spectroscopy (EDS, Ametek).

**Electrochemical measurement in bulk solution.** LSV of the wire-array (Supplementary Fig. 3) in bulk minimal medium was obtained using a typical three-electrode set-up on Gamry 1010B potentiosystem setup. The wire array was used as working electrode, a piece of silver paint-coated ITO glass was used as reference electrode, same as the pseudo-reference electrode used in the electrochemical chamber. Pt wire was used as counter electrode. Air-argon-saturated minimal medium was prepared by bubbling air/argon in minimal medium for 30 min. Such minimal medium was used as electrolyte. The LSV was obtained by scanning from 1.0 to −0.5 V vs. RHE at the speed of 5 mV/s\(^{1}3\).

**Construction of electrochemical setup on confocal microscope.** An electrochemical setup under a confocal microscope was constructed for both the experimental validation of O2 gradient and the characterization of electricity-driven N2 fixation (Supplementary Fig. 4). A three-electrode electrochemical configuration was fitted into a flow chamber with a chamber thickness of 200 µm (Fig. 2b and Supplementary Fig. 4). The wire array electrode constitutes the working electrode. An ITO-coated glass cover slip (1.5, 40 mm x 22 mm, SPI supplies), wet etched with 6 M HCl into two isolated electrodes, was used as the top cover of the chamber. One section of the ITO was sputtered with 7-nm Pt and used
as the counter electrode; the other one, coated with silver paint (Ted Pella, Inc) at the downstream direction, was designated as the Ag pseudo-reference electrode. Prior to the Ag pseudo-reference electrodes, 5 and for 30 min after the wire array were washed with 10% ethanol and dried at room temperature. The Ag pseudo-reference electrodes, prepared by allowing the use of objectives with working distance of 680 µm, were placed in a dish tor chemical operation. A syringe or peristaltic pump was employed to feed the liquid medium into the chamber at a fixed flow rate. A Gamry Interface 1010B was applied to characterize the electrochemical chamber equipped with a microwire array, which was sterilized by isopropyl alcohol before use. The microbes were allowed to associate with the wire array for one hour, before the chamber was rinsed with cell-free medium to remove excessive planktonic cells. The association was probably due to the fixation of any nitrogen and organic carbon sources, whose recipes are available in Supplementary Tables 5 and 6 for one hour. The microbes were allowed to associate with the wire array for one hour, before the chamber was rinsed with cell-free medium to remove excessive planktonic cells. The association was probably due to the fixation of any nitrogen and organic carbon sources, whose recipes are available in Supplementary Tables 5 and 6.

Characterization of the Ag pseudo-reference. To characterize the electrode potential vs. SHE, and test the stability of pseudo-reference electrodes, prepared by Ag paste, cyclic voltammetry was conducted in a standard Zobell’s solution[40] using our Ag pseudo-reference electrodes before and after the 120-h operation as the working electrodes. In each experiment of cyclic voltammetry, the device is exposed to Zobell’s solution, which is composed of 3.3 mM KFe(CN)6, 3.3 mM K3Fe(CN)6, and 0.1 M KC1[41]. Cyclic voltammograms were recorded at a scan rate of 20 mV s−1 using the pseudo-reference electrode as the reference, a 3.2 mm-diameter glassy carbon electrode as working electrode, and a Pt wire as the counter electrode (Supplementary Fig. 13). A minimal amount of time for the exposure of Zobell’s solution was ensured so that the exposure per se will not alter the redox potential of the pseudo-reference electrodes. As the Zobell’s solution is known to have a redox potential of ≈ 0.43 V vs. SHE[41], the potentials of pseudo-reference electrode were obtained after determining the mid-point potential of the redox couple in the cyclic voltammograms. Representative cyclic voltammograms before and after (Supplementary Fig. 13, black line, mid-point potential = 113 mV vs. reference) and after (Supplementary Fig. 13, red line, mid-point potential = 117 mV vs. reference) 120-h operation displayed minimal differences in the mid-point potential of Fe(III)/Fe(II) redox couple, indicating that the pseudo-reference electrodes maintain a stable 0.31–0.32 V vs. SHE over an extended period of time.

Experimental validation of O2 gradients. In situ phosphorescence and lifetime imaging of Ru(phen)3Cl2 was employed in the microwire electrode to validate the proposed O2 gradients (Fig. 2b). The phosphorescence was monitored at 570–650 nm by a 470-nm laser excitation, with a >20 immersion type objective lens (Leica ×20 HC PL APO IMM CS2 NA/0.75). 0.1 mM Ru(phen)3Cl2 (Sigma-Aldrich) in 1x phosphate buffer saline (PBS, pH 7.0). Supplementary Table 3) was being flowed through the chamber with a syringe pump (New Era Pump Systems, Inc.) at a flow rate of 2 ml min−1. The intensity of phosphorescence emission was collected by photon multiplier tube detectors (PMT). The data was acquired using Leica Application Suite X (Leica ×20 HC PL APO IMM CS2 NA/0.75). 0.1 mM Ru(phen)3Cl2 (Sigma-Aldrich) in 1x phosphate buffer saline (PBS, pH 7.0). Supplementary Table 3) was being flowed through the chamber with a syringe pump (New Era Pump Systems, Inc.) at a flow rate of 2 ml min−1. The intensity of phosphorescence emission was collected by photon multiplier tube detectors (PMT). The data was acquired using Leica Application Suite X (Leica Application Suite X). The spatial lifetime information was processed by the photon multiplier tube detectors (PMT). The data was acquired using Leica Application Suite X. The spatial lifetime information was processed by the time-correlated single photon counting (TCSPC) mode, taking a single-layered bacterial cross-sectional image on different z-position at a spatial resolution of 146 nm/pixel with a z increment of 1 µm. Such a fluorescence characterization leads to the determination the changes of microbial cell counts (δCC) in the wire array. Colorimetric assays that were used in environmental monitoring[42,43] were employed to yield the changes of total nitrogen content (ATN) and the changes of total organic carbon (TOC).

Estimation of bacterial growth within the wire array. We estimated the number of bacteria within the wire array using phosphorescence image obtained from confocal microscopy. For each x–y cross-sectional image, we estimate the total cell number by the total pixel number considered ‘light’, judged by a certain intensity threshold. The fluorescent image was processed using ImageJ. White noise was removed using ‘remove outliers’ command under the settings of 2.0 pixels radius and 30 as outlier threshold. The image was then transformed into a binary figure with the intensity threshold, in which “light” pixels are “1”s and “dark” pixels are “0”s. The sum of all “light” pixels in an image reveals how many pixels are repre- senting stained bacterial cells. The higher the sum, the more cells are in the image, as displayed in Fig. 3e. To quantify the absolute cell number, we used external standard. Briefly, a “standard image” was captured, using the same method and scan resolution as used in our microscopic observations, on a single-layered bacterial sample with each bacterium separated from each other. The bacterial number per pixel (pix/bacteria) was then obtained. For each x–y cross-sectional image, the sum of “light” pixels is divided by pix/bacteria to calculate the total number of bacteria in one image. This may lead to slight underestimation of bacterial numbers, due to the assumption that there was no overlap between bacteria in our captured images. To estimate the overall cell number in the three-dimensional space within the wire array, we assume that from the bottom of wire to the top of wire a new batch of bacteria will be observed with every 5 µm increase in z, as bacteria that were more than 5 µm away from focus plane will not appear in the cross-sectional image. Thus the overall number within the observed wire array would be Naverage × (15 µm), where is the length of wire and Naverage is the average bacterial number in x–y cross-sectional images acquired at different distances from wire bottom (z). As we noticed the bacterial growth at different z were not equal (as shown in Fig. 3e–g), the average cell number Naverage was taken by averaging the cell number in x–y images at z = 0, 10, 20, 30, 40, and 50 µm for a l = 50 µm wire array. The cell number in terms of density was acquired by dividing the overall cell number by the volume of imaged space. The increase in cell number was calculated by the difference of cell number after 5 days incubation and the cell number right after inoculation.

Total nitrogen (TN) and organic carbon determination. The general method to determine TN and total organic carbon (TOC) retained in biomass within wire array is multiplying the TN and TOC of a single bacterium by the number density of bacteria within the wire array, which is determined using method described in the method above. First, TN and TOC of bacterial culture with known cell number are determined by absorbance spectrometry using test kits (Hach, kit #10071 for TN
and Hach, kit #10627 for organic carbon). 5-day lithotrophically cultured bacterial cells are harvested from culture using centrifugation and re-suspended in nitrogen and organic carbon-free medium. The OD60 of bacterial suspension was adjusted to 0.7 before testing. The measuring procedure is briefly described here. For TN quantification, 2 mL of bacterial suspension is first digested using alkaline persulfate solution. This step quantitatively transforms all nitrogen species into nitrite. Then the solution was acidified to so that all nitrites turns to nitric acid. The nitric acid was then reacted with phenol, as this quantitative reaction yields yellow colored benzoquinone. The absorbance of generated benzoquinone at 410 nm is thus dependent on the concentration of nitric acid, which relates to the TN amount in bacteria. A calibration curve correlating the absorbance at 410 nm and TN concentration (Supplementary Fig. 17) was constructed using the kit on different standard nitrogen sample solutions (ammonium p-toluenesulfonate, Hach). For TOC quantification, 2 mL of bacterial suspension was first acidified and heated to remove inorganic carbonates. Then the solution was digested using persulfate to oxidize all organic carbon into carbon dioxide. The generated carbon dioxide was remove inorganic carbonates. Then the solution was digested using persulfate to oxidize all organic carbon into carbon dioxide. The generated carbon dioxide was then reacted with phenol, as this quantitative reaction yields yellow colored benzoquinone. The absorbance of generated benzoquinone at 410 nm is thus dependent on the concentration of nitric acid, which relates to the TN amount in bacteria. A calibration curve correlating the absorbance at 410 nm and TN concentration (Supplementary Fig. 17) was constructed using the kit on different standard nitrogen sample solutions (ammonium p-toluenesulfonate, Hach). The absorbance was measured using a Hewlett-Packard 8453 UV-vis spectrometer. Second, the number density of bacteria per OD60 was 2.8 × 10⁸ mL⁻¹ for X. autotrophicus (IMED) analytic flow cytometer, which was run on a BD LSRII (IMEX) analytic flow cytometer. The values of nitrogen content of each single cell were calculated using the overall TN or TOC of a culture with certain OD60 divided by the number of bacterium.

The TN and organic carbon for single bacterium cell (n = 3):

| Strain         | Total nitrogen (mg cell⁻¹) | Total organic carbon (mg cell⁻¹) |
|---------------|----------------------------|----------------------------------|
| X. autotrophicus | 1.5 ± 0.0 x 10⁻¹⁰          | 6.4 ± 0.3 x 10⁻¹⁰                |
| B. japonicum   | 4.4 ± 0.3 x 10⁻¹¹          | 1.6 ± 0.0 x 10⁻¹⁰                |

In order to validate the quantification method described above, when necessary, the microfluidic setup was carefully assembled. The microorganisms in the setup were carefully extracted by rinsing with minimal medium and mechanically scratching microwire array electrodes. As the microwire array electrodes were made of Si, SiO₂, indium tin oxide (ITO), and Pt, the mechanically detached particles of wire array will not interfere with the oxidative procedure for the measurement of TN in the microorganisms. Similar methods as mentioned above was applied on the detached microbes. The overall microbial nitrogen, after 120 h under E_app = −0.15 V vs. RHE in p = 15 µm microwire array, were 2.48 ± 0.52 and 0.503 ± 0.077 µg for X. autotrophicus and B. japonicum, respectively (n = 2), which corresponds to 992 and 201 mg L⁻¹ for X. autotrophicus and B. japonicum, respectively. These values are very close to the values measured using the general method mentioned above (975 and 196 mg L⁻¹ for X. autotrophicus and B. japonicum, respectively), validating our method of nitrogen quantification mentioned above.

The amount of free ammonia in the effluent of our N₂ fixation system is also determined by absorbance spectrometry using test kit (Hach, TNTplus 830). The flow cell with p = 15 µm microwire array is inoculated with bacteria and run under E_app = −0.15 V vs. RHE for 120 h, same as described in methods. The flow speed = 2 mL min⁻¹. A total of 20 mL of medium was used and the effluent was circulated in the system to accumulate released ammonia to a detectable level. The increase of ammonia nitrogen was determined by absorbance spectrometry (Hach, Method 10205). 5 mL of effluent was added into the reaction mixture of the kit. After thoroughly mixing, the mixture was reacted for 15 min. The final solution’s absorbance at 694 nm was used to determine the amount of ammonia. The increase of ammonia nitrogen found to be 0.017 ± 0.0 mg L⁻¹ in the effluent of B. japonicum (n = 2). In the effluent of X. autotrophicus, we did not find detectable amount of ammonia. Therefore, it is considered as 0 mg L⁻¹ in our calculations for activities and efficiencies. Comparing with the value of 187 mg L⁻¹ (B. japonicum) for the increased biomass nitrogen under the same condition, the free ammonia accounts for more than 40% of the TN fixed by B. japonicum.

N₂ fixation activity calculation. The activity of N₂ fixation displayed in Supplementary Table 1 is calculated in two units: mg N₂ per liter per hour (rN₂I) and mg N₂ per g dry weight biomass per hour (rN₂II). The experimental results for microbes cultured using p = 15 µm microwire array under E_app = −0.15 V vs. RHE for 120 h (Fig. 3d, entries 1 and 2) are used.

rN₂ = ΔTNbio × V_wire array + ΔTNmedium × V_medium / t

where ΔTNbio is the increase of TN in microbe cells within the array (5.7 × 10² and 1.9 × 10⁻¹⁰ mg L⁻¹ for X. autotrophicus and B. japonicum, respectively), V_wire array the volume of wire array (2.5 × 10⁹ L⁻¹), ΔTNmedium the increase of TN as ammonia in microbial growth medium (0 mg L⁻¹ for X. autotrophicus and 0.017 mg L⁻¹ for B. japonicum), V_medium the volume of medium circulated in the device during operation (20 mL), t the overall incubation time (120 h). From the equation we calculate the rN₂ values, which are 4.8 and 2.7 mg L⁻¹ h⁻¹ for X. autotrophicus and B. japonicum, respectively.

rN₂ was calculated using the sum of ΔTN and free nitrogen in effluent divided by the bacterial average dry mass (TNavg_average/15%):

rN₂ = ΔTNbio × V_wire array + ΔTNmedium × V_medium / (t/15% × 1000)

under the assumption that nitrogen constitutes 15% of bacterial dry mass. TNavg is the average nitrogen content in microbes over the growth time. We assume exponential growth and take the average of the biomass amount throughout the 120-h incubation as the TNavg (644 and 61 mg L⁻¹ for X. autotrophicus and B. japonicum, respectively). ΔTNbio is the increase of TN in microbe cells within the array (5.7 × 10² and 1.9 × 10⁻¹⁰ mg L⁻¹ for X. autotrophicus and B. japonicum, respectively), V_wire array the volume of wire array (2.5 × 10⁹ L⁻¹), ΔTNmedium the increase of TN as ammonia in microbial growth medium (0 mg L⁻¹ for X. autotrophicus and 0.017 mg L⁻¹ for B. japonicum), V_medium the volume of medium circulated in the device during operation (20 mL), t the overall incubation time (120 h). The values of TN of inoculum. The rN₂ values are 1.1 and 6.5 mg L⁻¹ h⁻¹ for X. autotrophicus and B. japonicum, respectively.

Calculation of Faradic efficiency (FE) and energy cost. We define FE as the percentage of electron that has been used to reduce N₂ and CO₂ to form microbial biomass and secreted ammonia. The FE is calculated as follows:

FE = ΔTNbio × V_wire array + ΔTNmedium × V_medium /

where ΔTNbio is the increase of TN in microbe cells within the array (5.7 × 10² and 1.9 × 10⁻¹⁰ mg L⁻¹ for X. autotrophicus and B. japonicum, respectively), V_wire array the volume of wire array (2.5 × 10⁹ L⁻¹), ΔTNmedium the increase of TN as ammonia in microbial growth medium (0 mg L⁻¹ for X. autotrophicus and 0.017 mg L⁻¹ for B. japonicum), V_medium the volume of medium circulated in the device during operation (20 mL), t the overall incubation time (120 h), the overall current density that pass through the wire array electrode during the 120 h operation of our hybrid system (ca. 80 µA cm⁻²), A, the projected area of wire array electrode (0.5 cm²), t the overall operation time (120 h, correspondingly 432,000 s), F, Faraday constant (96,485 C mol⁻¹), Mₐ and Mₖ, the molar atomic weight of N (14 g mol⁻¹) and C (12 g mol⁻¹), respectively. The FEs are calculated to be 1.4% and 0.42% for X. autotrophicus and B. japonicum, respectively (p = 15 µm; E_app = −0.15 V vs. RHE).

The energy cost of nitrogen fixation in terms of kJ/g nitrogen is estimated using the following equation:

Eₙ = (ΔTN_bio × V_wire array + ΔTN_medium × V_medium) / (1000 × A × U)

where Eₙ is the energy cost (kJ/g nitrogen), t the current density (ca. 80 µA cm⁻²), A, the projected area of wire array electrode (0.5 cm²), t the total incubation time with applied voltage (120 h, correspondingly 432,000 s), U the applied potential (2 V for water splitting), ΔTN_bio the increase of TN in microbe cells within the array (5.7 × 10² and 1.9 × 10⁻¹⁰ mg L⁻¹ for X. autotrophicus and B. japonicum, respectively), V_wire array the volume of wire array (2.5 × 10⁹ L⁻¹), ΔTN_medium the increase of TN as ammonia in microbial growth medium (0 mg L⁻¹ for X. autotrophicus and 0.017 mg L⁻¹ for B. japonicum), V_medium the volume of medium circulated in the device during operation (20 mL). Here we consider the nitrogen contents retained in biomass and secreted into the circulating medium. Following the procedure described above, the overall energy cost is 1.5 x 10⁷ and 2.6 x 10⁶ kJ/kg nitrogen for X. autotrophicus and B. japonicum strains, respectively (p = 15 µm; E_app = −0.15 V vs. RHE).

Data availability
The datasets generated or analyzed during the current study are available from the corresponding author upon reasonable request. The source data underings Figs. 1d, 2e, 2f, 3d, and 3e, and Supplementary Figs. 2, 3, 6, 9, 10, 12, 13, 15–18 are provided as Source Data file, which can be found following the url: https://doi.org/10.6084/m9.figshare.1189715v2.

Code availability
The code used in the current study are available from the corresponding author upon reasonable request.
