

2 **Light-dark changes in cytosolic nitrate pools depend on nitrate**
3 **reductase activity in *Arabidopsis* leaf cells**

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9 **Supplementary data (1 table and 3 figures)**

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11 **Table A.** Mean cytosolic nitrate activity in wild type and *nia1nia2* mutant epidermal
12 and mesophyll cells during light/dark transitions. Data was obtained using nitrate-
13 selective microelectrode recordings and this is the same data as shown in Figure 3.

Plant type	Cell type	Light treatment	Mean nitrate activity (mM)	
Wild type	Epidermal	Light	2.5	(±0.4)
		Dark	2.2	(±0.4)
	Mesophyll	Light	1.5	(±0.2)
		Dark	2.8	(±0.7)
<i>nia1nia2</i> mutant	Mesophyll	Light	3.6	(±0.6)
		Dark	3.9	(±0.6)

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15 Standard deviations shown in brackets, n > 5.
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17 **Figure A. Calibration response times obtained from a typical nitrate-selective**
18 **microelectrode.** This recording shows the chemical response times of the electrode as
19 the electrode tip was exposed to decreasing activities of nitrate from 100 mM to 10
20 mM to 1 mM to 0.1 mM nitrate. The differential response times of ion-selective and

2 reference barrels limit the time course of intracellular ion changes that can be
3 measured (Miller and Sanders, 1986; Sanders and Slayman, 1982).

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6 **Figure B. Additional examples of nitrate electrode recordings obtained during**
7 **light to dark and dark to light transitions from wild type epidermal and *nia1nia2***
8 **mesophyll leaf cells.** Bars indicate the time at which the light or dark (shaded)
9 treatment was applied. In a) and b) the electrode tip was located in the vacuoles of a
10 wild type plants. In c) and d) the tip was located in the cytosol of wild type and
11 *nia1nia2* plants respectively.

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14 **Figure C. Additional examples of nitrate electrode recordings obtained during**
15 **light to dark and dark to light transitions in *Arabidopsis* mesophyll leaf cells.** Bars
16 indicate the time at which the light or dark (shaded) treatment was applied. In
17 recording a) the light off event at 50.5 min generated a transient spike of
18 approximately 1min duration in the nitrate recording. This type of spike occurs more
19 rapidly than the chemical response time of the nitrate electrode (see Fig A) and so we
20 believe does not represent a true biological response. In the dark from 54 min onwards
21 a new steady higher cytosolic nitrate activity was established and this is the cellular
22 response we are reporting in this paper. Recording b) shows a dark to light transition
23 from another plant when this transient spike was not observed but a cytosolic nitrate
24 activity was established after about 5 min.

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Figure A: Calibration of a nitrate microelectrode

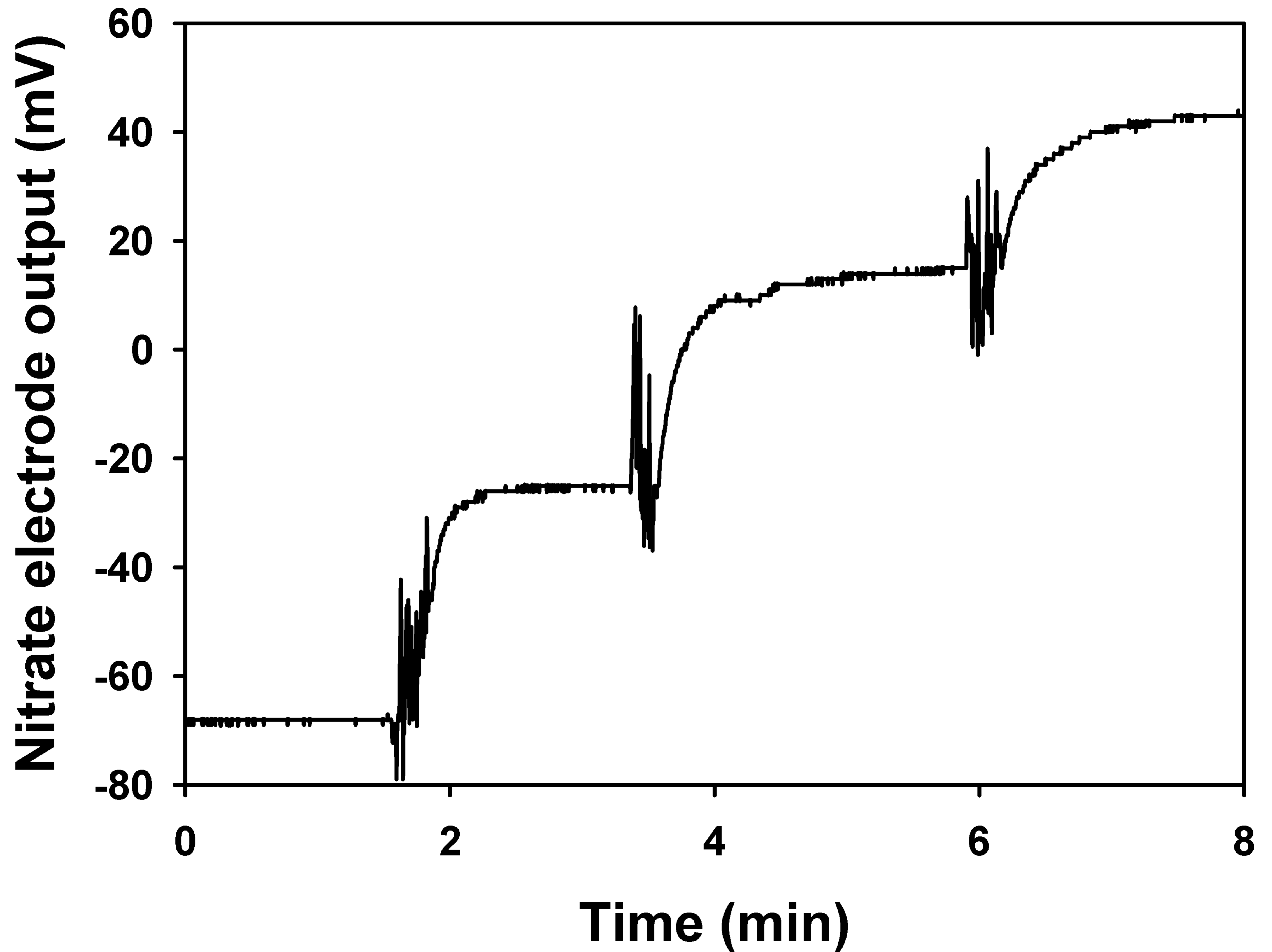


Figure B Supplementary data

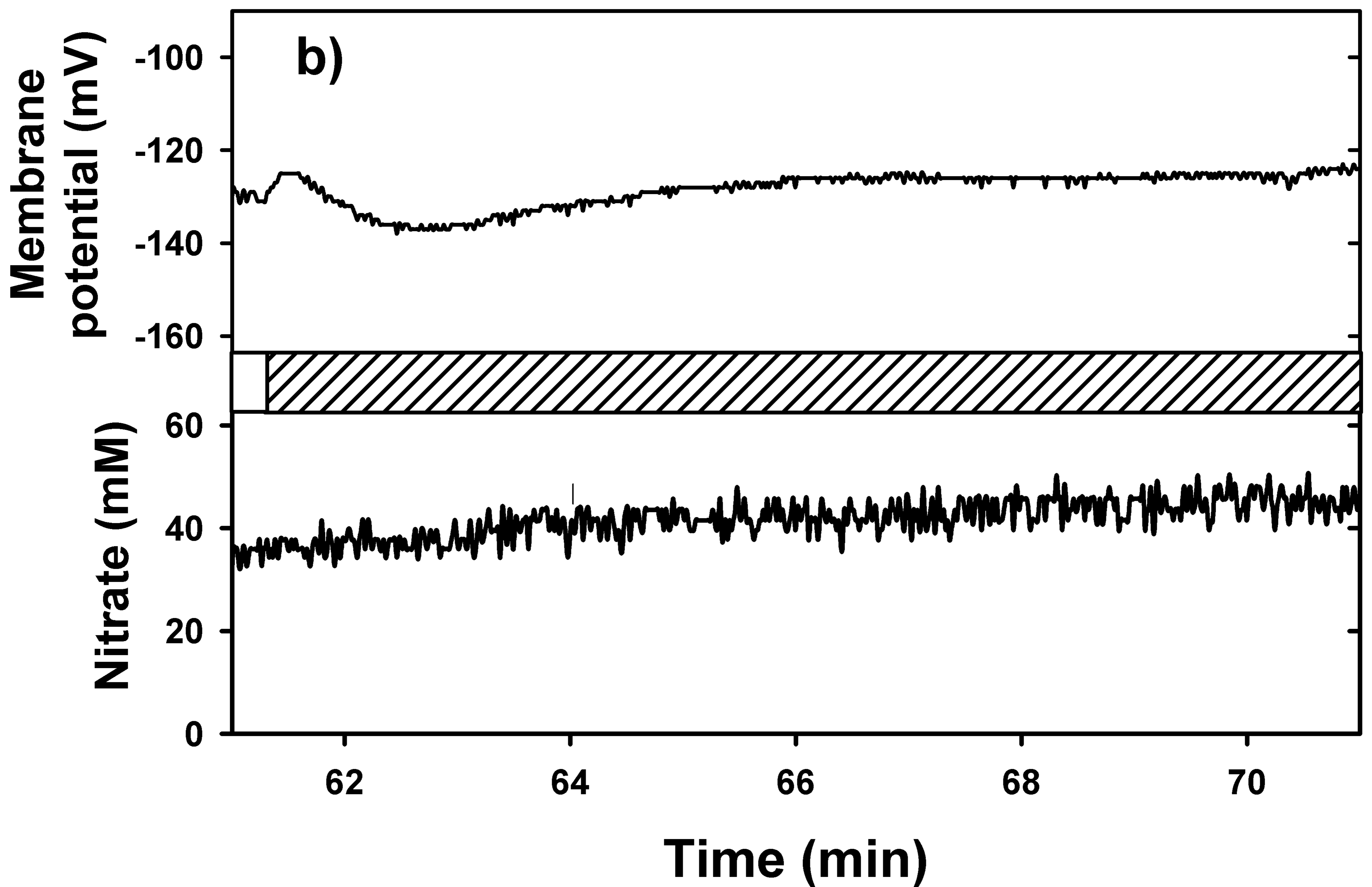
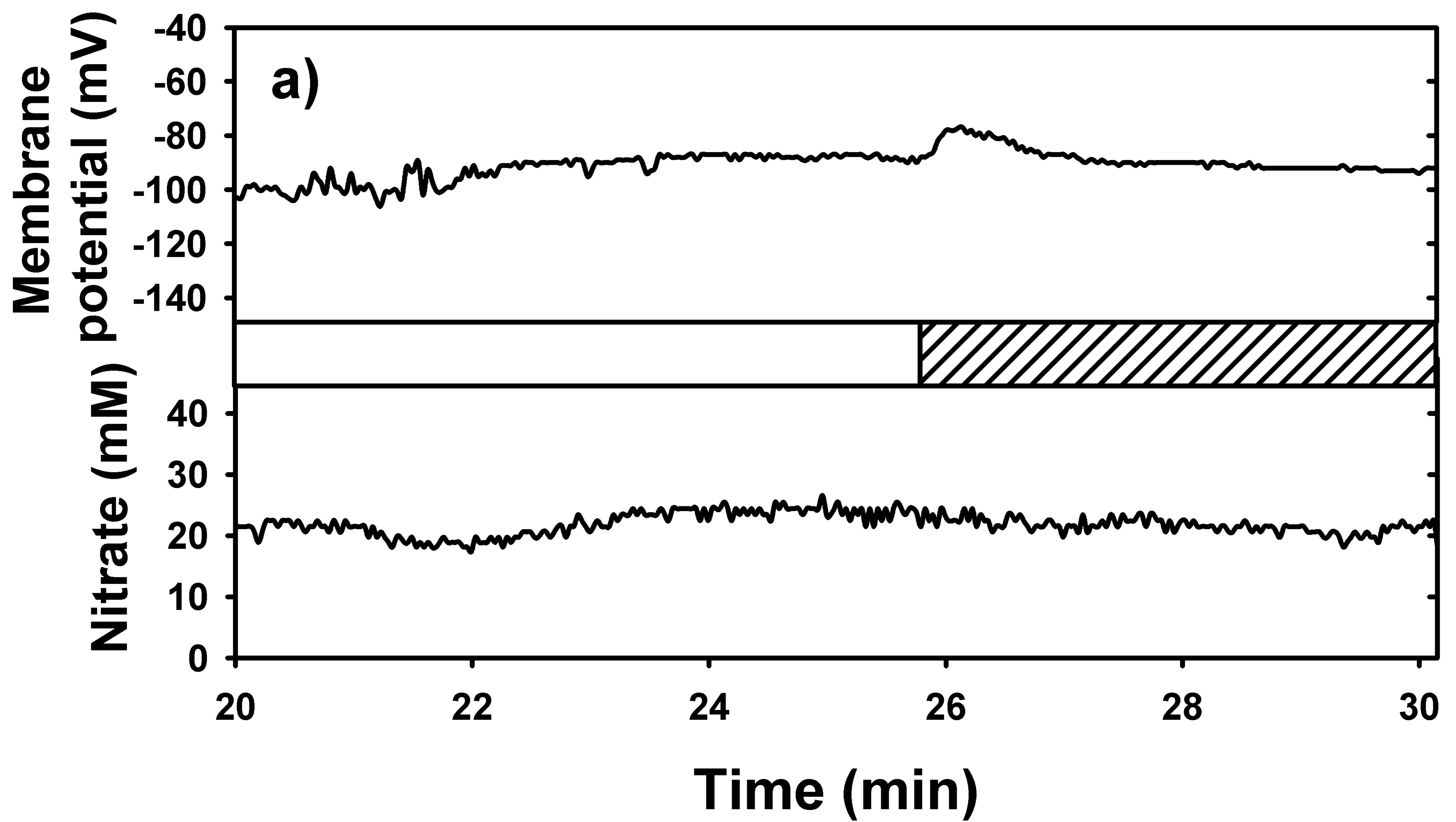
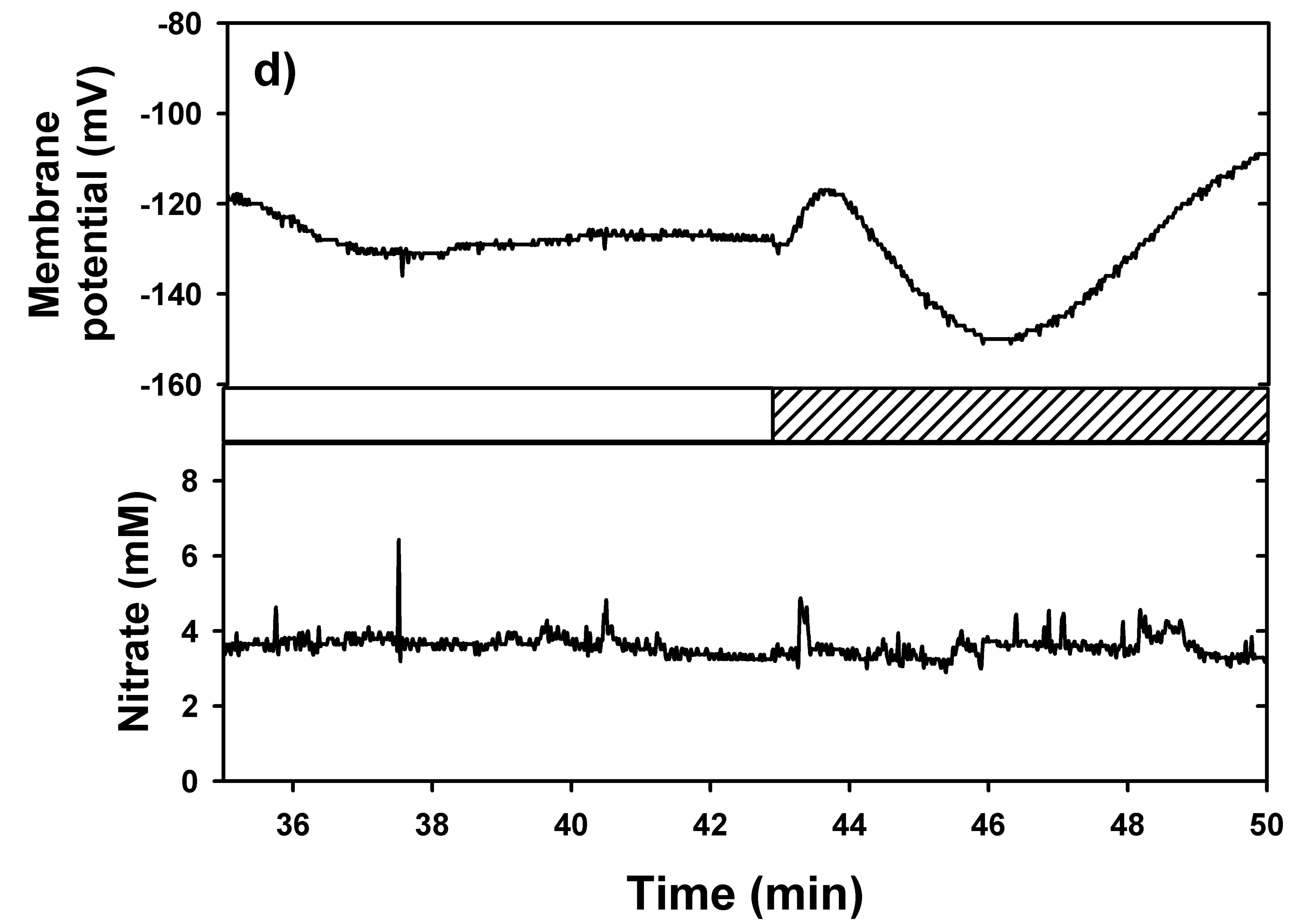
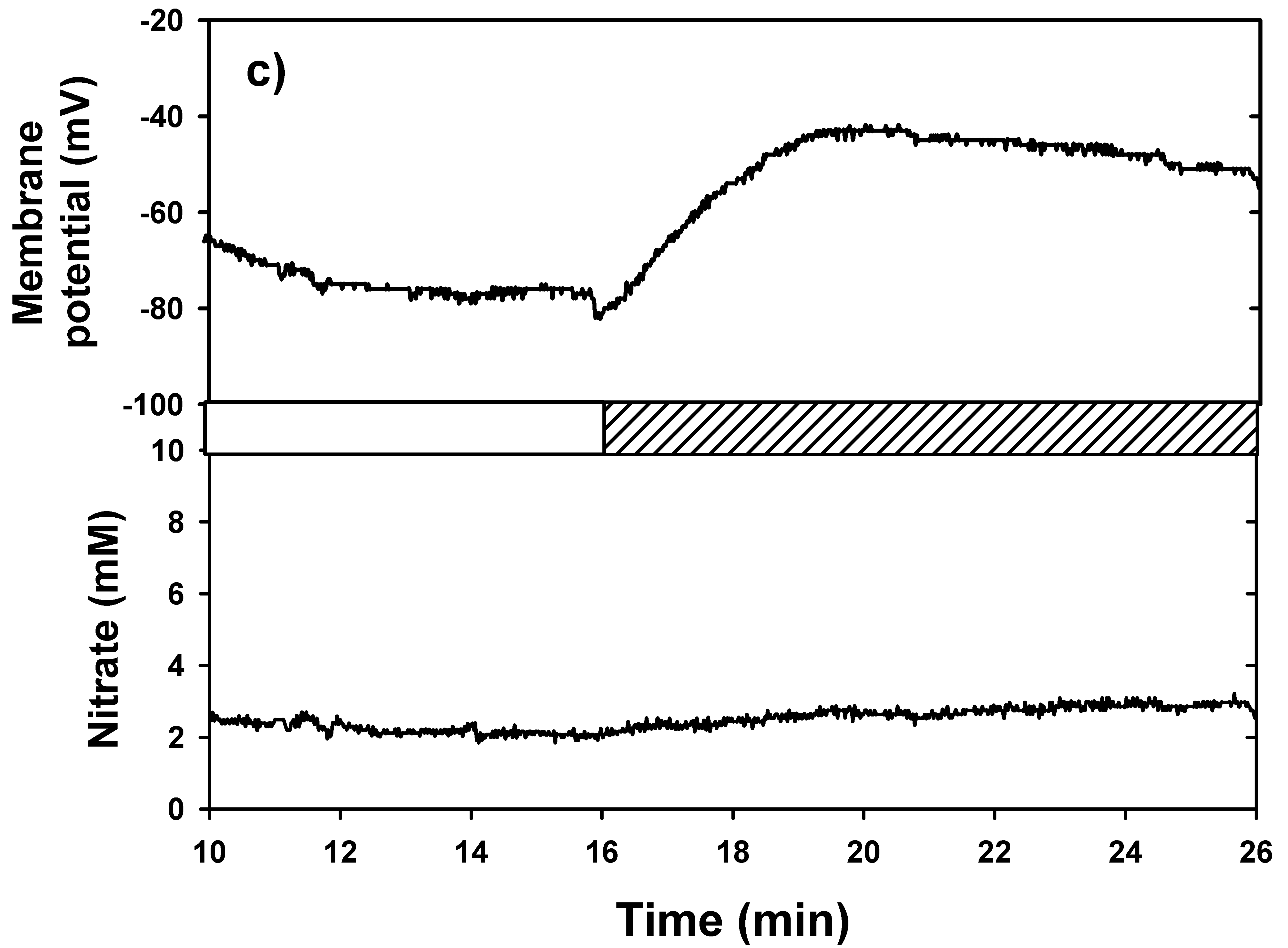


Figure B Supplementary data



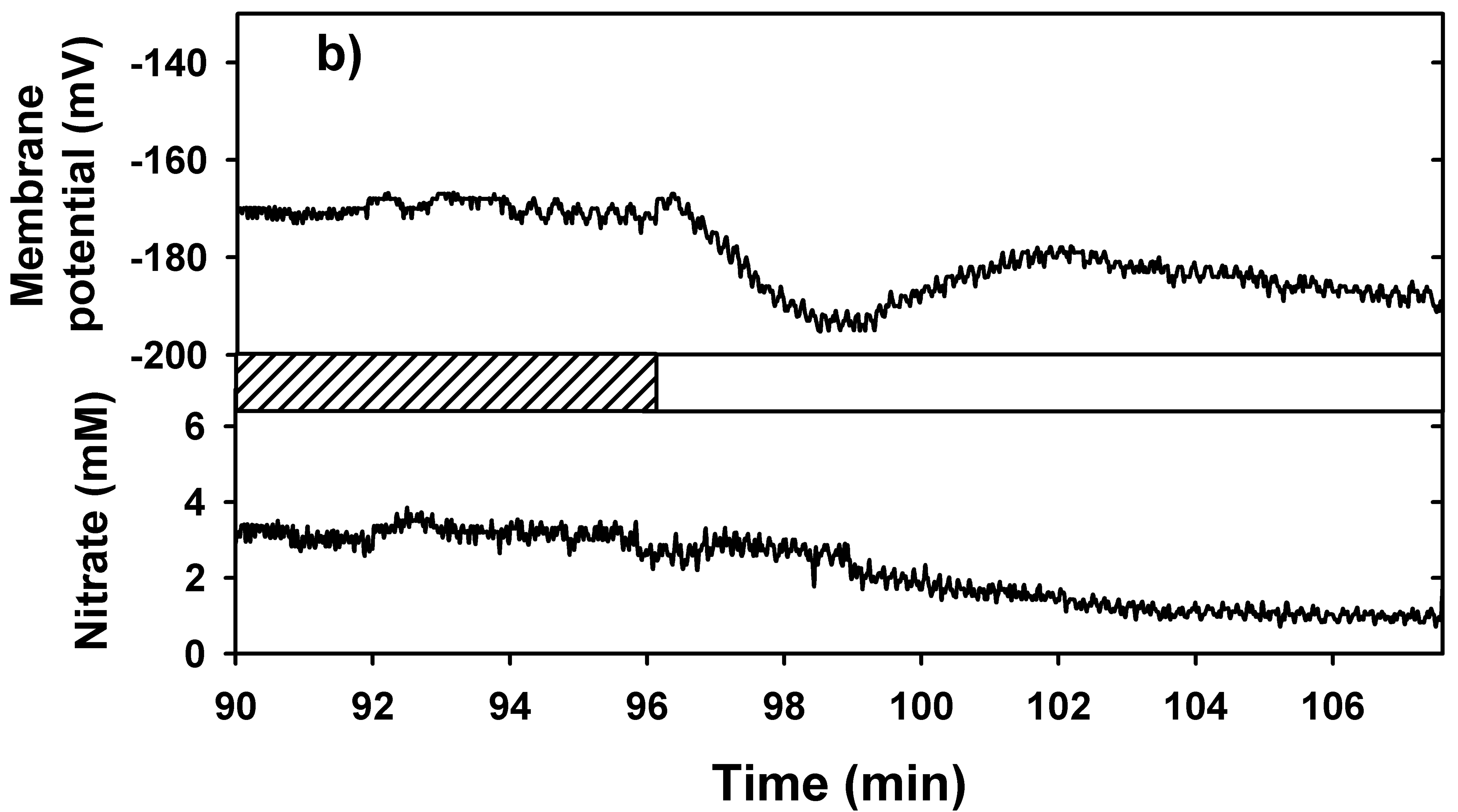
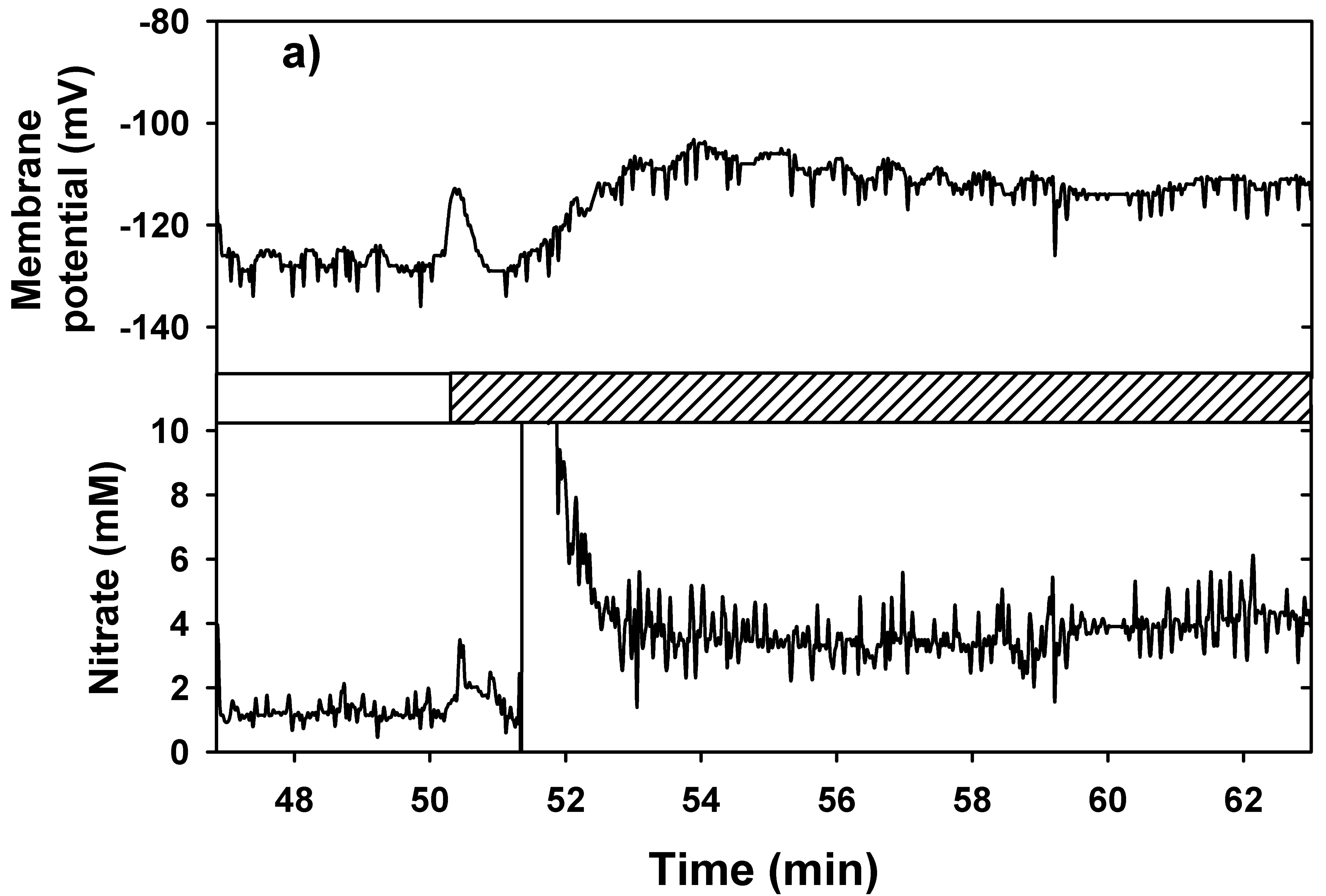


Fig C Supplementary data