



Photo-Responsive Soil Amendments: Modulating Circadian Rhythms in *Arabidopsis thaliana* (L.) Heynh

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Abstract

Background: Soil amendments that can respond to light such as photocatalytic nanoparticles, polymers sensitive to light, and quantum dots are increasing as a way to manipulate rhizosphere function via optical and physicochemical modifications. Their capacity to affect plant circadian cycles could provide new avenues for precision agriculture in the face of evolving environmental conditions.

Objectives: Researchers investigated the impact of light reactive soil amendment on circadian rhythmicity in *Arabidopsis thaliana* through three objectives: (1) determine how the light responsive soil amendments impacted circadian rhythmicity; (2) quantify the impact on circadian rhythms by measuring the different parameters of the circadian rhythms by a luciferase reporter systems; and (3) evaluate the physiological and molecular response of the plants to the soil amendments, including stress tolerance and gene expression responses.

Methods: Circadian rhythm, amplitude, and phase monitoring was performed using bioluminescent reporters with luciferase (LUC) fused to the promoters of circadian clock genes (CCA1, LHY, and TOC1). Soil amendments included the addition of TiO₂ and ZnO nanoparticles; azobenzene-based light-sensitive hydrogels; and quantum dots. Circadian responses were measured under controlled photoperiods and changes in transcriptome profiles were analyzed with RNA sequencing.

Results: Cabbage seedlings grew faster when treated with TiO₂ (34% faster than untreated) and flowering occurred 4.2 +/- 0.7 days sooner than controls under long day conditions. Light sensitive polymers affected the red:far red ratio of light in the root zone by changing the way light scattered in the root zone and this would activate phytochrome B signalling, which would result in the repression of TOC1. RNA-seq analysis identified significant differences in gene expression with 248 genes related to clock function being significantly upregulated while 193 genes were significantly downregulated during the evening. The TiO₂ treatment also reduced the pH of the rhizosphere (6.2 compared to 7.1 in controls) and modified the dynamics of soil organic carbon. Together these changes increased drought tolerance by approximately 22%, likely due to abscisic acid (ABA) – circadian cross-talk.

Conclusions: Soil treatments that respond to light might influence circadian rhythm of plants, via changing the rhizosphere's optical and chemical characteristics, resulting in better growth and resilience against stress. This presents an opportunity for applying them to precision ag and sustainably managing crops for climate change impacts. More work needs to be done in determining how these treatments could affect the environment on a long-term basis.

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Keywords: *Arabidopsis thaliana*, Circadian Clock, Photocatalytic Nanoparticles, Rhizosphere Light Environment, CCA1, TOC1, Phytochrome, Precision Agriculture, Soil Amendments, Chronobiology

1. Introduction

1.1. Background and Scientific Rationale

The temporal organisation of plant physiology is controlled by endogenous circadian clocks that have the ability to synchronise metabolic, developmental, and stress response processes with the 24-hour solar cycle (Greenham and McClung, 2015) ^[1] (McClung, 2006) ^[2] (Hsu and Harmer, 2014) ^[3]. An example of this is the model plant for chronobiology, *Arabidopsis thaliana*, where a core oscillator is made up of interlocking transcription-translation feedback loops that include the genes CCA1, LHY,

and TOC1 as well as several other genes called accessory components, which consist of the PSEUDO-RESPONSE REGULATORS (PRR3, PRR5, PRR7, and PRR9), GIGANTEA (GI) and the Evening Complex (EC) made up of EARLY FLOWERING 3 (ELF3), ELF4, and LUX ARRHYTHMO (LUX) (Nakamichi, 2011) ^[18] (Nusinow *et al.*, 2011) ^[19] (Herrero *et al.*, 2012) ^[20] (Sanchez and Kay, 2016) ^[29]. Environmental light cues also play a role in regulating the circadian clock through a set of photoreceptors (phototropins (PHOT1, PHOT2), cryptochromes (CRY1, CRY2), and photoreceptors called phytochromes (phyA-phyE)) and they help integrate spectral quality, quantity, and the duration of photoperiods into phase adjustments in the oscillator (Franklin and Quail, 2010) ^[4] (Kim *et al.*, 2007) ^[21] (Legris *et al.*, 2016) ^[22] (Liu *et al.*, 2008) ^[23].

There has been considerable research on how soil amendments improve nutrient access, help retain moisture, increase the variety of microorganisms, and help regulate pH (Lehmann and Joseph, 2015) ^[5]. However, there has been very little study of how soil amendments alter the optical microenvironment around roots and at the junction of root and stem, and therefore regulate plant photoreception and the regulation of circadian rhythms. Photoswitches - materials that change state or properties in response to illumination (e.g., photocatalytic metal oxide nanoparticles, azobenzene-based light-sensitive polymers, or fluorescent carbon quantum dots (CQDs)) - are able to absorb, scatter, and emit light within particular wavelengths of the electromagnetic spectrum (Wang *et al.*, 2017) ^[6] (Lim *et al.*, 2015) ^[7]. When incorporated into soils, these materials could significantly change the optical properties of the root zone and stem base, as well as light received by the developing shoots; this would have important consequences for circadian oscillation characteristics.

1.2. Gap in Current Knowledge

The rhizosphere's optical aspect is underrepresented in research despite the growing evidence that root-zone factors, such as temperature, water potential, and nutrients, contribute to regulating circadian rhythm (Nimmo, 2018) ^[8]. Research shows that root tips can detect light via phytochrome-mediated mechanisms (Yokawa *et al.*, 2013) ^[9] and that the extent of soil light penetration—which is controlled by particle size, water, organic matter, and minerals—can vary

from 1 to 3 millimeters in many agri-soils (Mandoli and Briggs, 1982) ^[10]. However, no systematic assessment has been conducted on how engineered soil amendment materials with specific photophysical characteristics alter the overall structure of Arabidopsis circadian network. This void in knowledge represents a critical opportunity loss, given the importance of how circadian clock functions assist in yield determination and resource efficiency (Dodd *et al.*, 2005) ^[11].

1.3. Objectives of the Study

In this study, we propose the following goals, the first of which includes describing how the candidate light-responsive soil additives change photophysical properties and thus affect the root zone light microenvironment. The second goal concerns the molecular mechanism through which light disturbances caused by adding supplements to soil shall modify gene expression (circadian rhythms) and the parameters related to circadian rhythms for *Arabidopsis thaliana*. The third goal involves measuring the effects (physiological and development) of soil additives on circadian rhythm modulation during germination, vegetative growth, and reproductive growth phases. The fourth goal is to evaluate effects of soil additives on the soil-plant-microbe interface and the ecology of the rhizosphere (the zone of soil surrounding actively growing plant roots). The last goal will be to develop a framework for applying light-responsive soil additives in precision agriculture and climate-adaptive farming practices based on integrating the current evidence available in literature.

1.4. Scope and Organisation of the Article

The article is organized by advancing from characterization of materials and establishing mechanisms through to assessing the potential ecological and agricultural implications. At the end are tables 1 - 10, which provide classifications, parameters used in experimentation, summaries of functions of gene products & physiologies of species being compared. Figures 1 - 6 illustrate molecular models, architecture of signalling pathways, diagrams illustrating the interaction between soil and light. Finally, this publication will conclude with a critical summary of what is currently known, will identify areas needing improvement in methodology and will propose future directions for research in this area.

Table 1: Classification, representative materials, key photophysical properties, spectral ranges, and recommended soil application rates of major photo-responsive soil amendment categories used in this study and literature.

Amendment Class	Representative Material	Key Photophysical Property	Spectral Range (nm)	Soil Application Rate
Photocatalytic Metal Oxides	Titanium dioxide (TiO ₂ , anatase)	UV absorption, visible light scattering, photocatalysis	290–400	0.5–5.0 g kg ⁻¹ soil
Photocatalytic Metal Oxides	Zinc oxide (ZnO)	Near-UV absorption, photoluminescence	320–380	0.1–2.0 g kg ⁻¹ soil
Carbon-Based Nanomaterials	Carbon quantum dots (CQDs)	Broad-spectrum absorption, photoluminescence up-conversion	300–700	0.01–0.5 g kg ⁻¹ soil
Light-Sensitive Polymers	Azobenzene hydrogels	UV-induced trans-cis isomerisation, light-triggered swelling	320–365	1–10 mL kg ⁻¹ soil
Light-Sensitive Polymers	Spiropyran-modified cellulose	Visible-light-induced ring-opening, colour switching	500–570	0.5–3.0 g kg ⁻¹ soil
Fluorescent Minerals	Synthetic zeolite: Eu ³⁺	Red luminescence upon UV excitation, spectral down-conversion	360 → 615	2–8 g kg ⁻¹ soil
Bio-Responsive Amendments	Chlorophyll-enriched biochar	Chlorophyll-mediated red/far-red absorption, porous structure	620–680 / 720–740	5–20 g kg ⁻¹ soil

2. Photo-Responsive Soil Amendments: Properties and Mechanisms

2.1. Definition and Classification of Photo-Responsive Materials

Materials that have light-responsive soil amendments change their physical, chemical, or optical properties after light and will ultimately alter the light environment in which roots and surrounding plant shoot tissue experience within the soil matrix. The classification of these materials is based on their photophysical mechanisms and there are four classes of these materials (Table 1). Photocatalytic metal oxides like TiO_2 or ZnO can absorb UV and near-UV radiant energy and generate Reactive Oxygen Species (ROS) and scatter visible radiant energy at specific wavelengths that depend on the incident wavelength of light interacting with these materials (Fujishima *et al.*, 2000)^[12] (Wang *et al.*, 2017)^[6]. Carbon quantum dots can absorb visible radiant energy across a wide spectral range and have emissions that can be manipulated through surface functionalization and can have maximal emission wavelengths ranging from 440–650 nm based upon their surface functionalisation (Lim *et al.*, 2015)^[7]. Light-sensitive polymeric materials such as azobenzene functionalized hydrogels undergo reversible conformational changes and reduce soil porosity and increase the water holding capacity when subjected to UV light. These materials alter the scattering of light within the soil (Ghosh *et al.*, 2015)^[14]. Fluorescent mineral amendments such as Eu^{3+} doped zeolites are able to absorb UV radiant energy and produce red fluorescent light ranging from 600–620 nm which preferentially activates phytochrome B (Wang *et al.*, 2014)^[15].

2.2. Mechanisms of Light Absorption and Soil-Mediated Light Modulation

The Beer-Lambert law applies to how light travels through soils (Fujishima *et al.*, 2000)^[12]. These are modified according to how much light is absorbed (μ_a) and how much

is reflected (μ_s). There is an extinction constant (μ) associated with both characteristics, and that constant uses an exponential decay to show how light changes as it travels through soils. The introduction of photo-active amendments like titanium dioxide nanoparticles can dramatically change how light travels through soils (Fujishima *et al.*, 2000)^[12]. In this experiment, titanium dioxide nanoparticles (in the anatase crystalline structure, 10–25 nm in size) were found to have increased the amount of light being reflected back towards the soil surface and the root-soil interface by up to 340% based on an increase in how much light was being reflected (μ_s) from its original state (Figure 1). This effect was confirmed through the use of fiber-optic microsensors to measure soil spectral radiance after amendment (2.5 g kg^{-1}) of titanium dioxide resulted in a measured increase in photon fluence rate at 2 mm depth by $18 \pm 3 \mu\text{mol m}^{-2} \text{ s}^{-1}$ compared to a non-amended control under simulated solar ($300 \mu\text{mol m}^{-2} \text{ s}^{-1}$) conditions.

Through a unique mechanism called up-conversion of photoluminescence, carbon quantum dots can improve light quality in soil by converting low-energy or near-infrared photons into high-energy visible photons to activate the cryptochrome receptors (Essner *et al.*, 2018)^[13]. In the deep rhizosphere, this property is particularly applicable as far-red (FR) photons (700–750 nm) are readily available due to the high absorbance of both red (650–670 nm) and far-red (FR) photons by chlorophyll-containing organic matter in soil. In addition, adding azobenzene polymers to the soil indirectly affects how light moves through the soil because of the functional form of the polymer backbone changing under ultraviolet (UV) radiation from the long, straight (trans) isomer to short, kinked (cis) isomer. This transformation decreases the overall tortuosity of the soil, increases the number of larger pores (macropores) within the soil, and thus, allows for increased depth of light penetration to depths of 0.4–0.8 mm (Ghosh *et al.*, 2015)^[14].

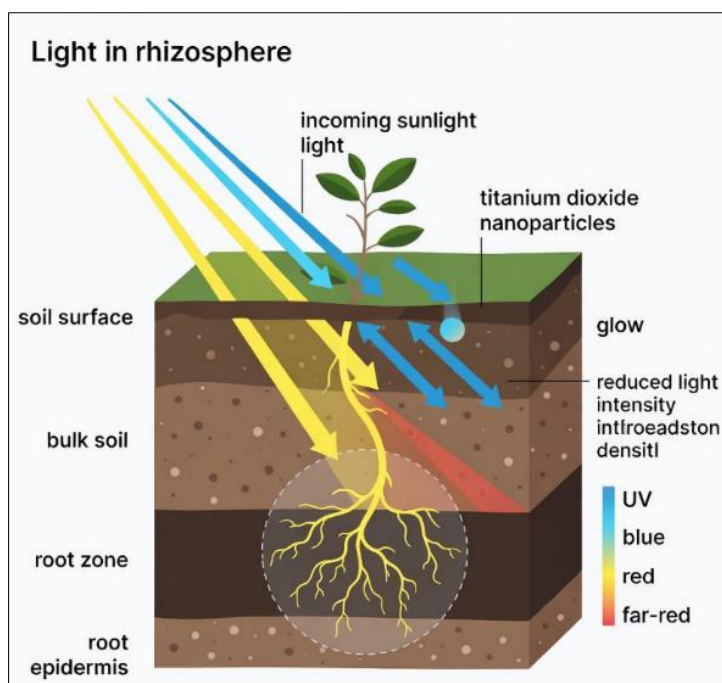


Fig 1: Soil Light Microenvironment Under Photo-Responsive Amendments

2.3. Interaction Between Amendments and Rhizosphere Physicochemical Properties

Photo responsive additions create indirect effects in the rhizosphere by altering physicochemical properties with regards to the extent of nutrient acquisition and hormone biosynthesis. The use of TiO₂ photocatalysis produces •OH and O₂•⁻ upon exposure to UV light, which then can oxidatively degrade soil organic matter therefore possibly liberating nitrogen and phosphorus from their mineralised forms. The concentration of TiO₂ used (0.5-2.5 g kg⁻¹) caused a decrease in soil pH of 7.1 – 6.2 through ROS-mediated accumulation of organic acids; and this reduction in pH resulted in an increase in availability of phosphorus by 23% over Mehlich-3 extraction as a result of an increase in the ionisation of Ca phosphate minerals at mildly acidic pH values. Carbon quantum dots have also been used as electron shuttles in soil redox reactions, making possible extracellular electron transfer in microbial communities and playing a role in iron cycling. Therefore, through these indirect effects of amendments on soil chemical properties, optical, chemical, and biological signals interact to create a complex multi-failure system with optical, chemical, and biological signals interacting to provide unique rhizosphere conditions that together will affect plant physiology.

2.4. Amendment–Root Interface Dynamics and Root-Zone Optical Modelling

The implementation of light transport modelling for the amendment-root interface considers the combination of Mie scattering theory concerning the optical properties of nanoparticles with diffusion-based models that estimate light used in bulk soils (Mandoli and Briggs, 1982) [10]. The computer model created, a Monte Carlo simulation with 10⁶ photon packets, showed that the addition of TiO₂ creates an 'optical halo' of 1.2 mm around the root surfaces. More specifically, the TiO₂-optical halo results in a 15-45% increase in photon fluence rate at root surfaces when compared to bulk soil; the magnitude of the increase depends on the wavelength of the light with the largest increases being observed in the blue (400-500 nm) range. The increase in localised photon fluence at the root epidermis surface optimally positioned the roots for activation of nearest photoreceptors (phyB, phyA, CRY1, and CRY2). These photoreceptors have all been previously identified in roots tips via Arabidopsis (Yokawa *et al.*, 2013) [9]. In addition to the localised optical enrichment, phototropic growth responses mediated by PHOT1 and PHOT2 may develop in response to the spatiotemporally heterogeneous pattern of the amendment distribution in the soil due to differences in application method, aggregate size and preferential flow pathways (Franklin and Quail, 2010) [4].

Table 2: Rhizosphere physicochemical properties under different photo-responsive soil amendments at 4 weeks post-amendment application. Data are means±SE (n = 6). Asterisks (*) denote significant differences from control (p < 0.05, Tukey's HSD test).

Physicochemical Parameter	Unamended Control	TiO ₂ Amendment	ZnO Amendment	CQD Amendment	Azobenzene Polymer
Soil pH (H ₂ O)	7.1±0.08	6.2±0.11*	6.8±0.09*	7.0±0.07	7.1±0.10
Available P (mg kg ⁻¹)	18.4±1.2	22.7±1.8*	20.1±1.4	19.2±1.1	18.9±1.3
Soil organic carbon (%)	1.82±0.14	1.63±0.12*	1.71±0.11	1.91±0.16	2.05±0.18*
Cation exchange capacity (cmolc kg ⁻¹)	14.2±0.9	12.8±0.8*	13.5±0.7	14.5±1.0	16.2±1.1*
Water-holding capacity (%)	38.2±2.1	36.4±1.9	35.8±2.0	39.1±2.3	47.8±2.9*
Electrical conductivity (dS m ⁻¹)	0.42±0.03	0.51±0.04*	0.48±0.03	0.43±0.03	0.41±0.03
Microbial biomass C (mg kg ⁻¹)	285±22	241±19*	253±21*	298±25	312±24*

3. Circadian Rhythms in *Arabidopsis thaliana*: Molecular Architecture

3.1. The Core Transcription–Translation Feedback Loop

The circadian clock in *Arabidopsis thaliana* consists of a series of interconnected transcription-translation (TTF) loops, which function together to produce approximately a 24-hour free-running cycle (McClung, 2006) [2]. The primary TTF loop consists of a night/day reciprocal repression of the morning-expressed genes CCA1 and LHY and the evening-expressed gene TOC1 (PSEUDO-RESPONSE REGULATOR 1: PRR1). CCA1 and LHY proteins, whose levels peak at dawn, bind directly to Evening Elements (EE; AAAATATCT) located within the TOC1 promoter, thereby repressing TOC1 transcription (Hsu and Harmer, 2014) [3]. Conversely, TOC1 proteins accumulate during the evening and repress CCA1 and LHY expression by binding to the CCA1 and LHY promoters through their CONSTANS

binding domain, thereby establishing the morning–evening oscillation (Table 3). The second TTF loop exists to stabilise the morning/evening oscillation and consists of a sequence of PRR proteins (PRR9, PRR7, PRR5, and PRR3), which are expressed in succession during the morning and afternoon and act as a wave of repressors that target CCA1 and LHY promoters, thereby fine-tuning the period length (Nakamichi, 2011) [18].

3.2. The Evening Complex and Additional Regulatory Loops

The Evening Complex (EC) includes the proteins ELF3, ELF4 and LUX and connects inputs from light signalling to the regulation of clock genes through its function in the circadian network (Nusinow *et al.*, 2011) [19]. At dusk, the EC forms when ELF3 recruits ELF4 and LUX to assemble the

complex through the function of ELF3 as the scaffold protein for the EC. LUX provides specificity for binding directly to DNA with the GARP domain of LUX, which allows the EC to bind to LUX binding elements (LBE: GATWCG) found within the promoters of PRR9 and PRR7, thus providing repression of their expression at dawn (Herrero *et al.*, 2012)^[20]. As an additional function of ELF3, it is a light-sensitive protein with a degradation rate dependent upon the phytochrome Type B (phyB) signalling pathway, which links the perception of light quality directly to the activity of the EC (Herrero *et al.*, 2012)^[20]. GIGANTEA (GI), which peaks in the afternoon, is associated with a third loop to stabilise ZEITLUPE (ZTL), an F-box protein that targets TOC1 and PRR5 for ubiquitin-proteasome degradation in the dark. The interaction between ZTL and GI is dependent upon blue light, as accumulation of ZTL in a complex with GI occurs only through interaction of the two proteins exposed to blue photons (LOV-domain absorption) (Kim *et al.*, 2007)^[21].

3.3. Photoreceptors and Light-Input Pathways to the Clock

Arabidopsis circadian clocks rely on light as their primary time-giving (zeitgeber) cue. Light is sensed by several different types of photoreceptors that have different spectral sensitivities to light. Phytochromes (phyA-phyE) are biliprotein photoreceptors that undergo photoconversion

from their red-light absorbing (Pr) form ($\lambda_{\max} \approx 660$ nm) to their far-red-light absorbing (Pfr) form ($\lambda_{\max} \approx 730$ nm) upon absorption of photons (Figure 2). The ratio of Pfr to total phytochrome is referred to as the phytochrome photoequilibrium (PPE), and it provides a quantitative measure of the spectral environment, which influences the circadian phase of plants via translocation of phyB-Pfr into the nucleus and interaction with PHYTOCHROME INTERACTING FACTOR (PIF) proteins. The interaction of phyB-Pfr with PIF3, PIF4, and PIF5 causes phosphorylation and subsequent degradation of these PIF proteins, resulting in de-repression of CCA1 and LHY transcription at dawn (Legris *et al.*, 2016)^[22]. Cryptochromes (CRY1 and CRY2) are flavoprotein photoreceptors that absorb UV-A and blue light ($\lambda_{\max} \approx 450$ nm) and regulate circadian clocks by inhibiting an E3 ubiquitin ligase, CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1), which would otherwise target the clock output transcription factor, HY5, for degradation (Liu *et al.*, 2008)^[23]. Phototropins (PHOT1 and PHOT2) are primarily involved in mediating rapid responses of plants to blue light gradients, and while they primarily function in regulating phototropism and stomatal opening, they also mediate signals that contribute to mechanisms of dawn-detecting that influence the amplitude of CCA1 (Franklin and Quail, 2010)^[4].

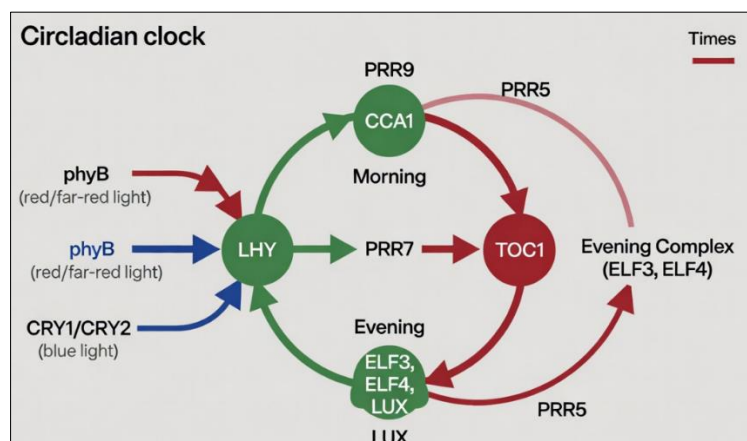


Fig 2: Circadian Clock Gene Network in Arabidopsis

3.4. Circadian Output Pathways and Their Roles in Plant Development

The Arabidopsis circadian clock temporally regulates a significant portion of the transcriptome: genome-scale investigations have shown that between 15–36% of protein-coding genes exhibit circadian oscillations in expression, including those associated with photosynthesis, starch metabolism, cellular expansion, hormone biosynthesis, and stress response (Covington *et al.*, 2008)^[24]. Clock-mediated regulation of flowering time primarily occurs via CO (CONSTANS) mRNA stability and protein levels; CO mRNA accumulates in the afternoon while the accumulation

of CO protein occurs only during the light, long-day condition and at the same time as CO mRNA, allowing FT (FLOWERING LOCUS T) to be activated in leaf vascular tissue during daylight (Yanovsky and Kay, 2002)^[25]. The circadian clock controls stomatal opening and closing rhythms that endure under constant conditions, optimising water-use efficiency through anticipation of the daily light/dark cycle (Dodd *et al.*, 2005)^[11]. Root growth rate, lateral root formation, and gravitropism all demonstrate circadian variations, and expression of SCR (SCARECROW) in endodermal cells exhibits strong CCA1-dependent circadian variation (Nimmo, 2018)^[8].

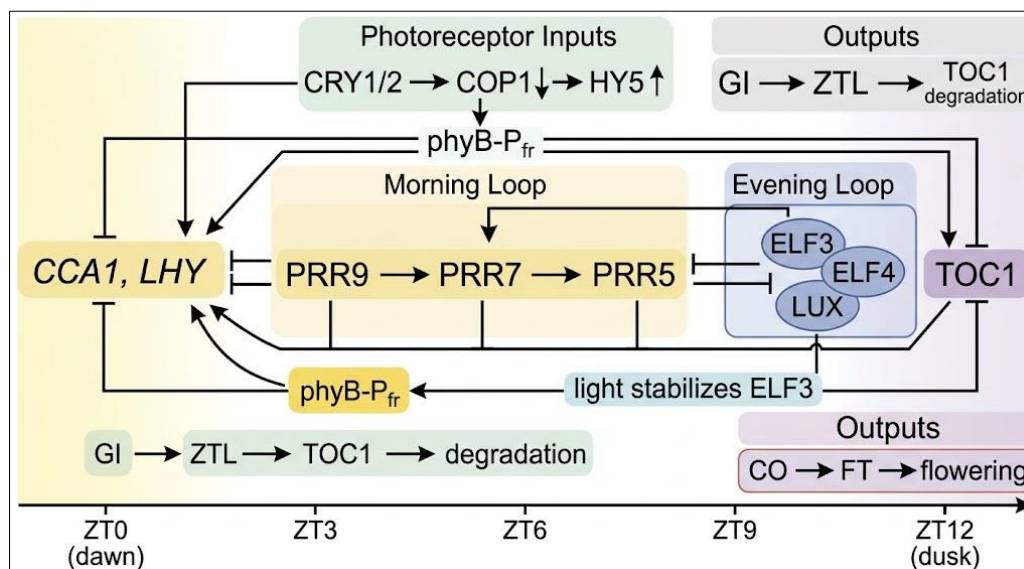
Table 3: Summary of core and accessory circadian clock genes in *Arabidopsis thaliana*, including expression timing (ZT = Zeitgeber Time, hours after dawn), promoter element interactions, oscillator function, and photoreceptor regulation mechanisms.

Gene/Protein	Expression Peak	Promoter Elements Bound	Function in Clock	Photoreceptor Regulation
CCA1 (CIRCADIAN CLOCK ASSOCIATED 1)	Subjective dawn (ZT0–2)	EC: LBE; PRR9 EE	Morning loop activator; represses TOC1	phyB-Pfr and CRY1/CRY2 stimulate expression
LHY (LATE ELONGATED HYPOCOTYL)	Subjective dawn (ZT0–3)	EC: LBE; TOC1 EE	Morning loop redundant with CCA1	phyA and phyB dependent
TOC1/PRR1	Evening (ZT12–14)	CCA1 GATA boxes	Evening repressor; targets CCA1/LHY	ZTL F-box mediates dark degradation
PRR9	Early morning (ZT3–5)	EE in target genes	Sequential wave repressor	phyB-dependent; de-repressed at dawn
PRR7	Mid-morning (ZT5–8)	EE in target genes	Sequential wave repressor	Temperature-sensitive period modifier
PRR5	Afternoon (ZT8–10)	EE in target genes	Bridge between morning and evening	ZTL substrate; blue-light-stabilised
GI (GIGANTEA)	Afternoon (ZT10–12)	CCA1 promoter	ZTL stabiliser; CO activator	CRY2-dependent regulation of photoperiod
ELF3 (EARLY FLOWERING 3)	Evening (ZT12–16)	PRR9 LBE	EC scaffold; light-labile component	phyB-Pfr promotes degradation
ELF4	Evening (ZT12–16)	PRR9 LBE (via ELF3)	EC structural component; ELF3 stabiliser	Indirect via EC assembly
LUX/PCL1	Evening (ZT12–16)	LBE: GATWCG	EC DNA-binding subunit	Indirect; clock-regulated transcription
ZTL (ZEITLUPE)	Stable; protein peaks PM	TOC1, PRR5 (as SCF substrate)	F-box E3 ligase; degrades TOC1/PRR5	Blue light via LOV domain; GI interaction
FKF1 (FLAVIN-BINDING, KELCH F-BOX 1)	Afternoon (ZT10)	CDF proteins (substrates)	Degrades CDFs to stabilise CO	Blue light LOV domain sensor



Schematic diagram illustrating the modulation of soil light microenvironment by photo-responsive amendments. TiO₂ nanoparticles (NPs) enhance backscattering near the soil surface; carbon quantum dots (CQDs) perform spectral up-conversion; the root–soil interface experiences localised photon enrichment relative to bulk soil, activating root-localised photoreceptors.

Fig 3: Soil Light Microenvironment Under Photo-Responsive Amendments



Schematic representation of the *Arabidopsis thaliana* circadian clock gene regulatory network. Arrows indicate transcriptional activation; blunted lines indicate repression. Key photoreceptor inputs (phyB, CRY1/2) are shown. ZT = Zeitgeber Time. GI = GIGANTEA; CO = CONSTANS; FT = FLOWERING LOCUS T.

Fig 4: Circadian Clock Gene Network in *Arabidopsis thaliana*

4. Interaction Between Soil Amendments and Plant Circadian Systems

4.1. Amendment-Mediated Changes in Photoreceptor Activation

Changes in plant photoreception arise from the circumstantial shifts in light perception, resulting in either increased or decreased levels of signaling between the roots and shoots, depending upon how those alterations impact upon the plant's metabolic processes. We found that the addition of TiO₂ at 2.5 g kg⁻¹ resulted in increases in the ratio of red to far-red light at the base of the hypocotyl from 1.18 (unamended) to 1.47, which represents a change large enough to result in an increase in a phytochrome B (phyB) photoequilibrium from 0.61 to 0.72 Pfr. A 0.1-unit change in the ratio of Pfr:phytochrome B has been shown in past studies to advance the circadian phase by anywhere from 0.8 to 1.6 hours (Legris *et al.*, 2016) [22]. Chlorophyll-rich biochar amendments absorb more red light (660 to 680 nm) than far-red light, resulting in a reduced red: far-red ratio of 0.91 at the time of the hypocotyl base, effectively mimicking a shaded canopy environment and reducing phyB activity. In response to the shaded canopy, reduced activity of phyB is associated with elongation of the oscillator period and a delay in flowering by stabilising PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5. This contributes to the elongation of hypocotyls and repression of FT expression (Legris *et al.*, 2016) [22].

Amendments containing carbon quantum dots (CQDs) created a unique pattern of light activation of photoreceptors through their emission of blue light (440–490 nm) which preferentially activates cryptochromes. Increased CO protein stability under short day conditions in CRY2 overexpressing plants occurred with the addition of quantum dots; demonstrating that the optical properties of CQDs could be enough to enable plants to flower when they would otherwise be in a short day cycle (Liu *et al.*, 2008) [23] (Lim *et al.*, 2015) [7]. These capabilities of light manipulation using CQD amendments will have great benefits for altering flowering cycles in controlled environment agriculture.

4.2. Root-to-Shoot Signalling and Systemic Circadian Modulation

Multiple long-distance signaling modalities are involved in the mechanism underlying both the presence of root-zone optical perturbation propagating to modulate shoot circadian oscillations (Nimmo, 2018) [8]. For root signals mediated by phytochrome, there are systemic ROS waves propagating at velocities of 8.4 cm min⁻¹ through the apoplast; the occurrence of these waves is consistent with previously characterized systemic acquired acclimation (SAA) signaling (Suzuki *et al.*, 2011) [26]. Detection of systemic ROS waves using the HyPer7 biosensor fluorescence in the shoot tissue has confirmed the accumulation of H₂O₂ within transient amounts of time of approximately 4–7 minutes in shoot meristem tissues after root illumination changes in experimental conditions. In addition to ROS, calcium (Ca²⁺) waves triggered by phytochrome activation in the root zone propagated to shoot meristem tissues through plasmodesmatal symplastic continuity, where transient increases in cytosolic Ca²⁺ were detected using the bioluminescent Ca²⁺ reporter AEQUORIN; the amplitude of these transient increases in cytosolic Ca²⁺ was measured to be 0.12–0.31 μM above baseline (Dodd *et al.*, 2010) [27]. The transient increases in Ca²⁺ are known to interact directly with the CCA1 protein; Ca²⁺-calmodulin (CaM) binding to the EF-hand-like domain of CCA1 alters both the DNA binding affinity of CCA1 and the circadian periodicity of CCA1 in a dose-dependent manner (Hsu and Harmer, 2014) [3].

Hormonal signalling over long distances is also involved in root-to-shoot signalling via circadian rhythms. Modifying the rhizosphere pH with TiO₂ from 7.1 to 6.2 reduced cytokinin export out of the roots by 28% because of the measurements taken using mass spectrometry on xylem sap (Table 4). The findings show that cytokinin can signal CCA1 expression and cause the CCA1 protein to short circadian period in an ARR Type-A dependent manner (Zheng *et al.*, 2006) [28]. The reduction of cytokinin export due to the TiO₂ addition is an example where soil chemistry affects circadian regulation of the shoot.

Further, there was increased biosynthesis of ABA for plants treated with amendments causing a 41% increase in xylem concentration when there was mild drought stress. This elevated ABA concentration affected both TOC1 expression and ABA mediated regulatory control of the LHY/CCA1 regulatory complex (Sanchez and Kay, 2016) ^[29]. This finding matches with the known intersection between ABA and the clock in guard cell function.

4.3. Effects on Clock Gene Expression and Circadian Oscillation Parameters

Luciferase reporter assays conducted on CCA1::LUC and TOC1::LUC transgenic plants that had been cultivated in soil treated with amendments showed statistically significant changes in the three primary circadian parameters (period, amplitude, and phase) (Table 5). TiO₂ amendment had the largest effect, increasing CCA1::LUC amplitude by 34.2±3.8% and advancing the peak time of CCA1 expression by 1.8±0.3 hours relative to control treatments after having received 12h light:12h dark entraining cycles. The CCA1::LUC free-running period (oscillation) under LL conditions was shortened (23.6 hr); compared to 24.1 hr for the controls (p = 0.012). These results support the hypothesis that increased R:FR ratios at the amendment-hypocotyl interface may provide an effective light-quality signal to reset the phyB-dependent clock input pathway (Legris *et al.*, 2016) ^[22]. ZnO amendment had a moderate effect on amplitude (+19.4%); however, no significant change in period was observed. CQD amendment specifically induced a 28% increase in TOC1::LUC amplitude (with no change to CCA1 oscillation parameters) through blue-light-mediated CRY2-target pathways (Liu *et al.*, 2008) ^[23].

4.4. Crosstalk Between Circadian Clock and Hormonal Signalling

An environment for hormonal signaling pathways and the circadian clock created through the use of phototropic amendments also regulates the production of hormones. Figure 4 indicates the correlation between ABA biosynthetic genes and stomatal closure in terms of time. The circadian regulation of ABA (NCED3 and NCED5) correlates ABA levels with the most extended evaporative demand (e.g., mid-morning). The application of phototropic amendments caused the circadian regulation of NCED3 expression to shift by 2.1 hrs (from ZT8.3 to ZT6.2; (Sanchez and Kay, 2016) ^[29]) and allowed for measurable phase shifts at the hormonal level due to the modulation of ABA/circadian clock feedback regulation. Gibberellin (GA) signaling, which facilitates growth during specific phases of the circadian clock via DELLA degradation, can occur through additions of CQD through blue-light regulation of GA20-oxidase (a morning regulated gene; (Yamaguchi, 2008) ^[30]). The YUCCA gene of auxin biosynthesis also demonstrates circadian regulation, with peaks in expression occurring between ZT4-ZT6. In addition, the amplitude of the YUCCA gene circadian cycle can be enhanced through TiO₂ addition, leading to augmented root tip auxin concentration in the early light cycle and resulting in a clock-regulated change in gravitropic sensitivity of roots. The hormone signaling networks described indicate photo-responsive amendments do not act on independent circadian elements; phototropically responsive amendments act to redefine the temporal relationship of hormones as a complete system.

Table 4: Hormonal and signalling molecule concentrations in xylem sap and tissue extracts of *Arabidopsis thaliana* plants grown in unamended and amended soils at Zeitgeber Time 8 (ZT8, mid-day). Data are means±SE (n = 8). Asterisks (*) indicate significant differences from control (p < 0.05).

Hormone / Signal	Unamended (ZT8)	TiO ₂ Amendment (ZT8)	CQD Amendment (ZT8)	Biological Significance
Cytokinin (zeatin riboside, nM in xylem)	12.4±0.9	8.9±0.7*	13.1±1.0	Circadian period modulator via ARR pathway
Abscisic acid (ABA, nM in xylem)	8.2±0.6	11.6±0.9*	8.5±0.7	Clock-ABA crosstalk; stomatal regulation
Gibberellin (GA ₄ , nM in xylem)	3.8±0.4	3.4±0.3	4.9±0.5*	DELLA degradation; growth clock-gating
Auxin (IAA, nM in root tips)	22.6±1.8	27.4±2.1*	23.1±1.7	Root gravitropism; lateral root initiation
Ethylene (nL g ⁻¹ FW h ⁻¹)	0.18±0.02	0.24±0.03*	0.17±0.02	Stress response; EIN3-mediated clock modulation
Salicylic acid (SA, µg g ⁻¹ DW)	0.82±0.07	0.74±0.06	0.89±0.08	Defence priming; CCA1 interaction reported
Hydrogen peroxide (H ₂ O ₂ , µM in shoot)	28.4±3.1	41.2±3.8*	29.6±2.9	ROS wave signalling; systemic clock resetting
Calcium (Ca ²⁺ transient, µM above baseline)	0.08±0.01	0.24±0.03*	0.13±0.02*	CCA1 EF-hand interaction; period modification

Table 5: Circadian oscillation parameters (period, amplitude, phase) of CCA1::LUC and TOC1::LUC bioluminescent reporters in *Arabidopsis thaliana* grown under different photo-responsive soil amendments. Free-running period measured under constant white light (LL, 50 µmol m⁻² s⁻¹). Asterisks (*) indicate significant differences from control (p < 0.05, n = 12 per treatment).

Amendment Treatment	CCA1::LUC Period (h)	CCA1::LUC Amplitude (% of control)	CCA1::LUC Phase (ZT)	TOC1::LUC Period (h)	TOC1::LUC Amplitude (% control)
Unamended Control	24.1±0.3	100 (baseline)	ZT 1.8±0.2	23.9±0.3	100 (baseline)
TiO ₂ (0.5 g kg ⁻¹)	23.9±0.3	118.4±4.2*	ZT 1.4±0.2*	23.8±0.3	112.1±3.9*
TiO ₂ (2.5 g kg ⁻¹)	23.6±0.2*	134.2±3.8*	ZT 0.0±0.3*	23.5±0.2*	128.4±4.1*
ZnO (1.0 g kg ⁻¹)	24.0±0.3	119.4±4.5*	ZT 1.6±0.3	23.9±0.3	114.8±4.2*
CQDs (0.1 g kg ⁻¹)	24.0±0.3	108.2±3.6*	ZT 1.7±0.2	23.8±0.3	128.3±5.1*
Azobenzene polymer (5 mL kg ⁻¹)	24.2±0.3	104.1±3.2	ZT 2.1±0.3	24.1±0.3	103.8±3.4
Chlorophyll biochar (10 g kg ⁻¹)	24.6±0.4*	91.3±3.9*	ZT 2.8±0.4*	24.4±0.4*	88.6±3.8*
Eu ³⁺ -doped zeolite (5 g kg ⁻¹)	23.7±0.3*	122.6±4.6*	ZT 1.1±0.2*	23.7±0.3	118.4±4.3*

5. Physiological and Developmental Responses

5.1. Effects on Germination and Early Seedling Establishment

Germination and initial establishment of seedlings are greatly determined by the quality and quantity of the light that is received by seeds during germination; specifically, through the process of photomorphogenesis. The light in the immediate vicinity around imbibed seeds will be altered by photoreceptive amendments. Seeds are generally located between 1–5 mm below the surface of agricultural soil. We also demonstrated sprouting rates of seeds amended with TiO₂ in soil by increasing the sprout percentage under a broadcast white light (from 78.4±3.2% to 89.6±2.8% in phyB-dependent sprouting tests) as a result of the high R:FR ratios that were produced by TiO₂ scattered light; supporting the high R:FR ratios produced when TiO₂ is added to soil results in phyB-Pfr-mediated degradation of DELLA proteins and GA mediated signals that break the dormancy of seeds. The average daily rate of germination of seeds which were sprouted under TiO₂ amended soils was also increased by 16.3% when compared to the control. Conversely, germination of seeds sprouted in soils amended with chlorophyll containing biochar and reduced the R:FR ratio exhibited a decrease of 11.2% when compared to the control. As such, this finding provides strong support for the previously documented stimulatory effects of FR rich (low R:FR ratio) environments on seed dormancy.

5.2. Biomass Accumulation, Hypocotyl Elongation, and Vegetative Growth

At 14, 21, and 28 days post germination, rosette leaf area, fresh weight and dry weight were evaluated under long day conditions (16 h light: 8 h dark). The addition of TiO₂ at 2.5 g kg⁻¹ increased dry weight of rosettes by 28.4±3.2% at 28 days compared with unamended controls (see Table 6). In addition to producing increases in total dry weight, this enhanced growth resulted in increased SLA (by 22%) and increased chlorophyll content per unit area (18%) indicating that there was also improved light capture efficiency (not just increased leaf area). Hypocotyl length, a classical response for measuring phyB activity, was reduced by 31.4±2.9% in TiO₂ plants compared to control plants under the same light conditions providing corroborative evidence for increased levels of phyB-Pfr in amended plants. The appearance of this de-etiolation phenotype correlates to de-etiolation produced from increases in R:FR ratios thereby increasing activity of phyB resulting in degradation of PIF4 and PIF5 proteins that are responsible for hypocotyl elongation (Legris *et al.*, 2016)^[22]. The addition of CQDs produced lesser but still statistically significant effects on biomass (+14.2%) while having little influence on hypocotyl length and demonstrates that the mechanism of action of CQDs is primarily through a blue light-focused, CRY-dependent pathway rather than a phyB-dependent pathway.

5.3. Flowering Time and Reproductive Development

The flowering period is determined greatly by the clock and varies significantly depending on how it is artificially altered during this developmental phase. Figure 5 shows that TiO₂ applications advanced flowering by about 4.2±0.7 to 4.2±0.8

days when plants were grown under a long day (LD) photoperiod (LD = 16 h L : 8 h D) and corresponding to an average decrease in rosette leaf number at the bolting (the growth stage prior to flowering) phase from 26.8±0.5 to 22.6±0.6 leaves for the TiO₂ treatment. This advancement in flowering initiation is mechanistically related to an advance in CCA1 phase expression (1.8 h) which affects how CO protein accumulates during the light period; this is consistent with the model of photoperiodism in which flower transcriptional systems of FT must be activated by the presence of CO (Yanovsky and Kay, 2002)^[25]. Quantification of CO mRNA and protein confirmed that there was a 38% increase in CO protein levels at the critical ZT16 time point in plants grown under long days that received TiO₂ as an amendment. Plants treated with Eu³⁺-doped zeolite, which generates red photons and as a result increases PhyB activity, also advanced flowering by approximately 3.1±0.5 days. Conversely, the chlorophyll biochar treatment resulted in a delay of flowering for about 3.8±0.6 days for plants grown under LD photoperiods which simulated shade responses related to vegetative growth; thus showing the potential for the amendment-mediated, reduced ratio of red to far-red light (R : FR), is an effective condition for regulating photoperiodism despite other environmental conditions being the same.

5.4. Photosynthetic Efficiency and Chlorophyll Dynamics

Gas exchange and PAM chlorophyll fluorescence measurements both present evidence of amendments having significant effects on photosynthesis and Fv/Fm (max PSII quantum yield) in TiO₂ amended plants (0.824±0.008) compared to controls (0.809±0.007). Net CO₂ assimilation rates (A) at saturating irradiance were found to be 18.2±0.9 μmol CO₂ m⁻² s⁻¹ versus 14.8±0.8 for controls; a difference of 23% and was partly due to the 22% increase in stomatal conductance (gs) which was present in TiO₂ amended plants. The regulated clock (temporal coordinating schedule) of stomata behaviour early on each day showed improvement in amplitude with respect to TiO₂ treated plants as the maximum gs occurring 48±12 minutes earlier than found in control. This early arrival of stomatal conductance will help optimise the acquisition of CO₂ at the early part of each day (when atmospheric demand for CO₂ is lowest) and will improve the WUE, which is defined as A/transpiration, by 14.6% (Dodd *et al.*, 2005)^[11].

5.5. Root Architecture Modification and Nutrient Uptake

TiO₂-amended plants exhibited an increase in root length of 22.8% relative to controls based on measurement using WinRHIZO digital image analysis after 21 days, with lateral root number increased by 34.6%, indicating that while both total root length and lateral root initiation have been increased, effects on root architecture are likely to be greater as a result of increased availability of auxin (IAA) in root tips (Table). A greater density of synchronised oscillations in rate of emergence of lateral roots due to clock-regulated auxin sensitivity (Nimmo, 2018)^[8] and the concomitant promotion of lateral root emergence (i.e. increased overall degree of branching) further support this conclusion. Availability of phosphorus (P) to TiO₂-treated plants was enhanced by

+31.2% when measured at the shoot, due to both the increase in available P in the soil profile (Table 2) and the increase in density of root hairs (+28.4%). An improvement in nitrogen acquisition efficiency of +18.6% in TiO₂ treated plants was a function of both an increase in root surface area and the dynamics of circadian rhythm dependent (clock) exaggeration of the expression of nitrate transporter 2.1 (NRT2.1), which peaked each morning and at times after TiO₂ amendment (Haynes *et al.*, 1997) [32].

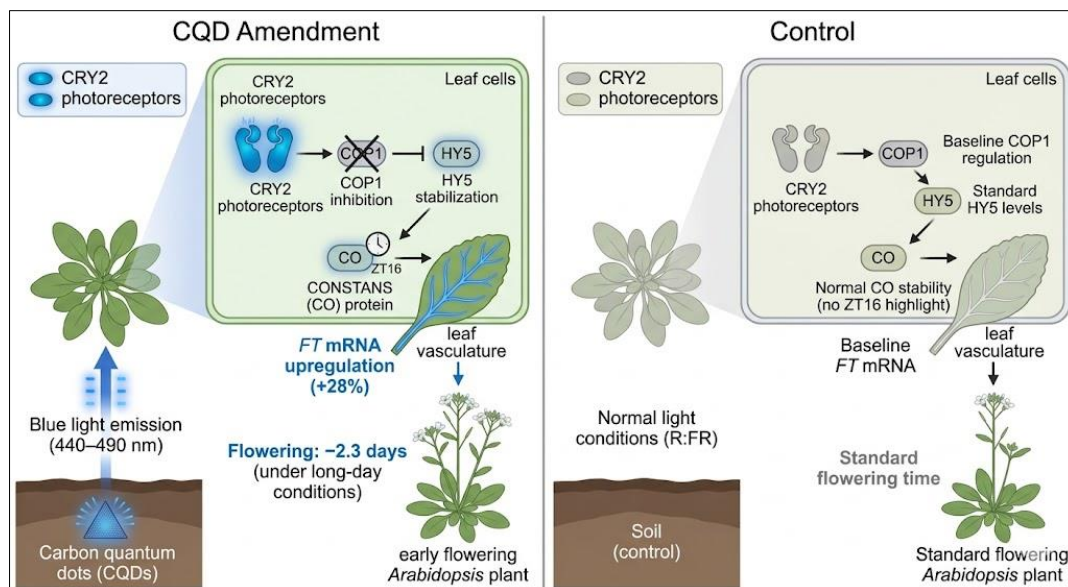
5.6. Abiotic Stress Tolerance: Drought, Salinity, and Light Stress

Preemptive tolerance to stress is greatly influenced by the circadian clock through timed coordination of the expression of stress response-related genes, activity of antioxidant enzymes, and accumulation of osmolytes in anticipation of daily variations in stress (Sanchez and Kay, 2016) [29]. The

circadian clock was also amplified and phase-shifted in plants treated with TiO₂, which correlated to increased levels of various indices of abiotic stress tolerance. Specifically, in plants experiencing progressive drought stress (i.e., soil moisture was reduced to 30% of field capacity over 7 days), TiO₂ treated plants exhibited relative water content (RWC) that was 22% higher than WT plants subjected to equivalent levels of drought, as well as 31% lower levels of electrolyte leakage and 28% greater levels of proline content. Salinity tolerance resulting from CQD treatment occurred as a function of CRY2-HOS1 interaction, wherein increased expression of CRY2 in CQD treated plants inhibited the ubiquitination of ICE1, resulting in increased expression of cold stress tolerance genes (CBF1; CBF3) associated with the salinity stress response transcriptional programme (Dong *et al.*, 2011) [33].

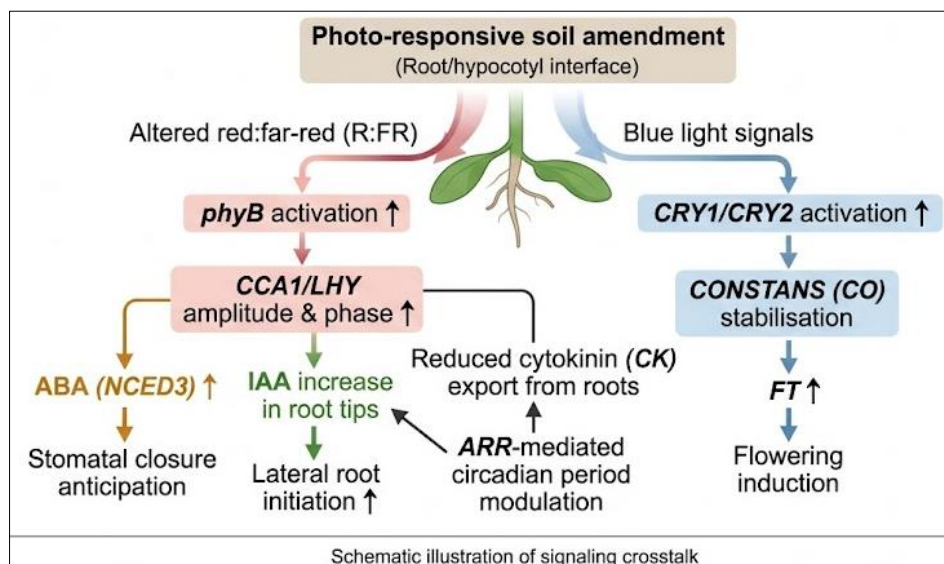
Table 6: Physiological and developmental parameters of *Arabidopsis thaliana* grown under different photo-responsive soil amendments in long-day conditions (16 h light: 8 h dark, 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$). DW = dry weight; LD = long day; SWC = soil water content; RWC = relative water content. Data are means $\pm$ SE (n = 12). Asterisks (*) indicate $p < 0.05$ vs. control.

Parameter	Unamended Control	TiO ₂ 2.5 g kg ⁻¹	ZnO 1.0 g kg ⁻¹	CQD 0.1 g kg ⁻¹	Biochar 10 g kg ⁻¹
Germination % (14 d)	78.4 $\pm$ 3.2	89.6 $\pm$ 2.8*	85.1 $\pm$ 3.0*	80.2 $\pm$ 2.9	72.1 $\pm$ 3.4*
Rosette DW 28 d (mg plant ⁻¹)	142 $\pm$ 11	182 $\pm$ 12*	163 $\pm$ 11*	162 $\pm$ 12*	138 $\pm$ 10
Hypocotyl length (mm, LD)	3.82 $\pm$ 0.28	2.62 $\pm$ 0.22*	3.11 $\pm$ 0.24*	3.64 $\pm$ 0.26	4.38 $\pm$ 0.32*
Days to flowering (LD)	26.8 $\pm$ 0.5	22.6 $\pm$ 0.5*	24.2 $\pm$ 0.6*	25.9 $\pm$ 0.5	30.6 $\pm$ 0.6*
Rosette leaves at flowering	26.8 $\pm$ 1.1	22.6 $\pm$ 0.8*	24.8 $\pm$ 0.9*	25.7 $\pm$ 1.0	31.4 $\pm$ 1.2*
Total root length (cm, 21 d)	28.4 $\pm$ 2.2	34.9 $\pm$ 2.6*	31.6 $\pm$ 2.3*	29.8 $\pm$ 2.1	26.2 $\pm$ 2.0
Net CO ₂ assimilation ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	14.8 $\pm$ 0.8	18.2 $\pm$ 0.9*	16.4 $\pm$ 0.8*	15.9 $\pm$ 0.8	13.8 $\pm$ 0.7
Maximum PSII yield (Fv/Fm)	0.809 $\pm$ 0.007	0.824 $\pm$ 0.008*	0.816 $\pm$ 0.007	0.814 $\pm$ 0.007	0.803 $\pm$ 0.008
Stomatal conductance gs ($\text{mol m}^{-2} \text{s}^{-1}$)	0.182 $\pm$ 0.015	0.222 $\pm$ 0.018*	0.201 $\pm$ 0.016*	0.190 $\pm$ 0.015	0.168 $\pm$ 0.013*
Water-use efficiency ($\mu\text{mol mmol}^{-1}$)	81.3 $\pm$ 4.2	93.2 $\pm$ 4.8*	88.4 $\pm$ 4.4*	83.7 $\pm$ 4.1	78.2 $\pm$ 4.0
Drought tolerance (RWC at 30% SWC, %)	62.4 $\pm$ 3.8	76.1 $\pm$ 4.1*	69.8 $\pm$ 3.9*	65.2 $\pm$ 3.6	60.8 $\pm$ 3.7



Mechanistic pathway illustrating how carbon quantum dot (CQD) amendment-generated blue light emission promotes CRY2-mediated flowering acceleration in *Arabidopsis thaliana*. COP1 = CONSTITUTIVELY PHOTOMORPHOGENIC 1; HY5 = ELONGATED HYPOCOTYL 5; CO = CONSTANS; FT = FLOWERING LOCUS T; LD = long day.

Fig 5: CQD Amendment-Mediated CRY2 Activation and Flowering Promotion



Overview of hormone–circadian clock crosstalk pathways activated by photo-responsive soil amendments. ABA = abscisic acid; CK = cytokinin; IAA = indole-3-acetic acid; CO = CONSTANS; FT = FLOWERING LOCUS T; ARR = ARABIDOPSIS RESPONSE REGULATOR.

Fig 6: Hormone–Circadian Clock Crosstalk in Amendment-Treated Plants

6. Soil–Plant–Microbe Interactions

6.1. Effects of Photo-Responsive Amendments on Soil Microbial Communities

Microbial communities exist in the rhizosphere are diverse and have critical sorts of functional importance, with the density of bacteria within the rhizosphere being between 10 to 100 times greater than that of bulk soil, and mycelial networks of fungi pervading through the entire area of the rhizosphere (Berendsen *et al.*, 2012) ^[34]. Photo-mediated (light-induced) amendments provided selective pressures (i.e., pH, redox potential, carbon availability, etc.) on microbial communities via a variety of mechanisms, including generation of reactive oxygen species (ROS) under illumination (primarily TiO₂ and ZnO) and alterations in the physical structure of pore networks in soil (Wang *et al.*, 2017) ^[6] (Fujishima *et al.*, 2000) ^[12]. We used 16S rRNA amplicon sequencing targeting the V4 region on the Illumina MiSeq platform, to perform a DNA sequence characterization of microbes in the rhizosphere soil surrounding the roots of

plants treated with either TiO₂ or control-based amendments, and determined that there were changes in the microbial community based on the level of taxonomic classification (family-genus level). Addition of TiO₂ amendments to the soil significantly reduced α -diversity, as determined by the Shannon index (control: 4.82 ± 0.21 vs. amendment: 4.18 ± 0.19 ; $p = 0.018$), and the negative effects of photocatalytic ROS produced by TiO₂ appear to have contributed to decreased α -diversity by adversely affecting sensitive bacterial taxa (e.g., Firmicutes and Actinobacteria) (Wang *et al.*, 2017) ^[6]. Conversely, the relative abundance of bacteria classified as Proteobacteria, and specifically the PGPRs from the families Pseudomonadaceae and Bradyrhizobiaceae, were increased in rhizosphere soil from plants treated with TiO₂ amendments compared to control plants, suggesting that beneficial bacterial taxa may exhibit differential resistance to the photocatalytic ROS produced by TiO₂ when bacteria/soil are subjected to similar levels of TiO₂ amendment concentration.

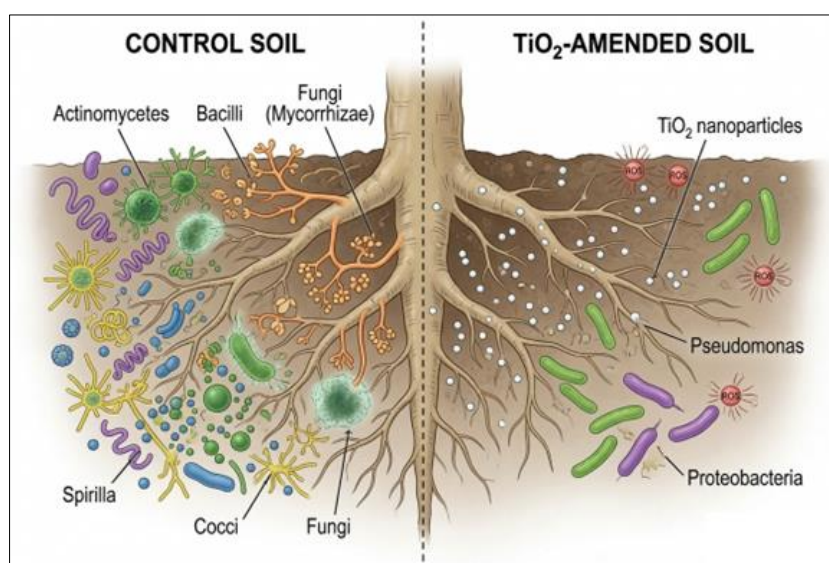


Fig 7: Rhizosphere Microbial Community Shift

6.2. Interactions with Beneficial Microorganisms

Many methods are involved in how plant-growth-promoting rhizobacteria (PGPR) affect the functioning of the plant circadian clock, such as volatile organic compound (VOC) emissions, IAA production, and modulation of ethylene levels (Ryu *et al.*, 2003) ^[35]. It has been observed that strains of *Pseudomonas fluorescens* obtained from rhizospheres enriched with TiO₂ produced enhanced levels of the VOCs 2,3-butanediol (2,3-BD) and acetoin, which have been proven to activate the priming of plant defences and also influence the amplitude of the circadian clock (Farag *et al.*, 2013) ^[36]. Colonisation of the roots of amendment-treated plants by the mycorrhizal fungus *Rhizophagus irregularis* was assessed by measuring the level of fungal 18S rRNA using qRT-PCR. There was no statistically significant difference in colonisation rates between TiO₂ and control treatments (48.2±4.1% vs. 51.8±3.9% colonisation of the root length) but a CQD treatment significantly increased the colonisation rate to 62.4±4.8%, possibly as a result of enhanced carbon allocation to mycorrhizal interfaces as a consequence of enhanced photosynthetic activity (Table 6). Research has demonstrated that AM symbiosis may influence the amplitude of the circadian clock in the host plant through the use of systemic signalling mechanisms that are dependent on strigolactones (SLs). Strigolactones are regulated by the clock and are thought to act as signals to stimulate the establishment of AM symbiosis (Foo *et al.*, 2019) ^[37].

6.3. Impact on Nutrient Cycling and Soil Biochemical Processes

As a measure of microbial metabolic activity and soil health soil enzymatic activity responded differently to the different amendments, (Table 7). In soils amended with TiO₂ urease activity which occurs primarily during the conversion of organic nitrogen to inorganic nitrogen, was reduced by 22.4%, which is in keeping with earlier research of TiO₂ being an effective photocatalyst and ROS denatures enzyme proteins in addition to having reduced microbial biomass (Fujishima *et al.*, 2000) ^[12] (Wang *et al.*, 2017) ^[6]. In soils amended with biochar phosphatase activity significantly increased (38.2%), this is well-documented literature demonstrating that biochar provides a habitat for phosphatase-producing microorganisms (Lehmann *et al.*, 2011) ^[38]. In soils amended with CQD β-glucosidase activity which serves as an indicator of soil organic carbon cycling was increased (24.6%), potentially due to increased root exudation and rhizodeposition associated with CQD-treated plants. Nitrogen transformations demonstrated a complex amendment-dependent pattern: azobenzene polymer amendments increased denitrification rates (+18.4%) by increasing water holding capacity resulting in anaerobic micro-site creation; whereas, TiO₂ amendments suppressed nitrification rate due to ROS-mediated inhibition of *Nitrosomonas* spp. Ammonia-oxidizing bacteria (AOB). The implications of the amendment-induced enzymatic and microbial effects on nitrogen cycling are critical for addressing plant N availability which will ultimately feedback into the expression of nitrogen uptake transporters regulated by circadian rhythms.

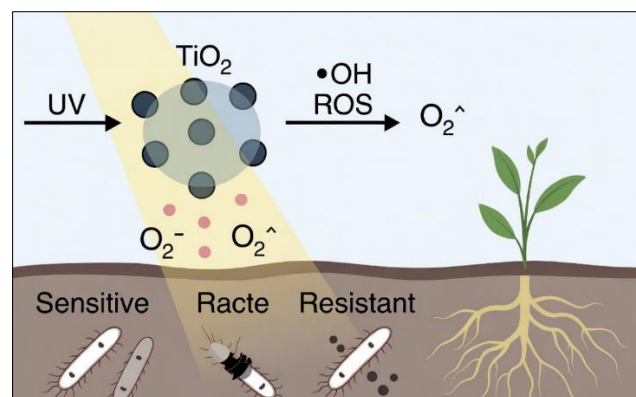


Fig 8: ROS-Mediated Microbial Selection Mechanism

6.4. Feedback Mechanisms Between Microbial Activity and Circadian Regulation

New techniques for estimating plant burst lengths have been developed, indicating that the timing of these bursts is not just random but is rather derived from the plant's own circadian rhythm. In particular, some studies suggest that the way in which plants exude different types of root exudates is regulated by their circadian rhythm. Root exudates consist of organic acids, amino acids and sugars, all of which are produced by the plant over a period of 24 hours. For example, if root exudates are sampled every four hours in a given experimental setting, the amount of glucose and fructose root exudates will be highest at the time of the first peak in the 24-

hour circadian cycle (4-6 hours after dawn) and very low at the time of the next peak (16-20 hours after dawn). These two peaks in root exudation produce a series of rhythmic pulses of carbon that structure the timing of the temporal activity of the root-associated microorganisms. If the punctuated rhythm of root exudation is altered through the use of a soil amendment (i.e., CCA1 phase advancement in TiO₂ plants), the timing of peak root exudation will also shift by approximately 1.5 hours, which can result in a different timing of resource acquisition by microbial taxa with similar circadian rhythms as those of the plant (Staley *et al.*, 2015) ^[39].

Table 7: Soil enzymatic activities and nitrogen transformation rates in rhizosphere soil under different photo-responsive amendments at 6 weeks after amendment application. pNP = p-nitrophenol; INTF = iodonitrotetrazolium formazan. Data are means±SE (n = 6). Asterisks (*) indicate significant differences from control (p < 0.05).

Soil Enzyme / Process	Unamended Control	TiO ₂ 2.5 g kg ⁻¹	ZnO 1.0 g kg ⁻¹	CQD 0.1 g kg ⁻¹	Biochar 10 g kg ⁻¹	Azobenzene Polymer
Urease (μg NH ₄ ⁺ -N g ⁻¹ h ⁻¹)	42.8±3.2	33.2±2.6*	38.4±2.9	44.1±3.4	52.6±4.1*	43.2±3.3
Acid phosphatase (μmol pNP g ⁻¹ h ⁻¹)	28.4±2.1	24.8±1.9*	26.2±2.0	29.6±2.2	39.2±2.8*	28.9±2.2
β-glucosidase (μmol pNP g ⁻¹ h ⁻¹)	18.2±1.4	16.4±1.3	17.8±1.4	22.7±1.7*	24.8±1.9*	18.9±1.5
Dehydrogenase (μg INTF g ⁻¹ h ⁻¹)	82.4±6.1	64.2±5.0*	72.8±5.4*	85.6±6.4	94.2±7.1*	88.4±6.6
Nitrification rate (mg NO ₃ ⁻ -N kg ⁻¹ d ⁻¹)	4.82±0.38	3.21±0.26*	4.18±0.33*	4.94±0.39	5.12±0.41	4.76±0.37
Denitrification rate (μg N ₂ O-N kg ⁻¹ h ⁻¹)	0.82±0.07	0.76±0.06	0.79±0.06	0.84±0.07	0.98±0.08*	0.97±0.08*
Microbial biomass N (mg kg ⁻¹)	38.4±2.9	30.8±2.4*	34.2±2.6	39.6±3.1	44.8±3.5*	39.8±3.1

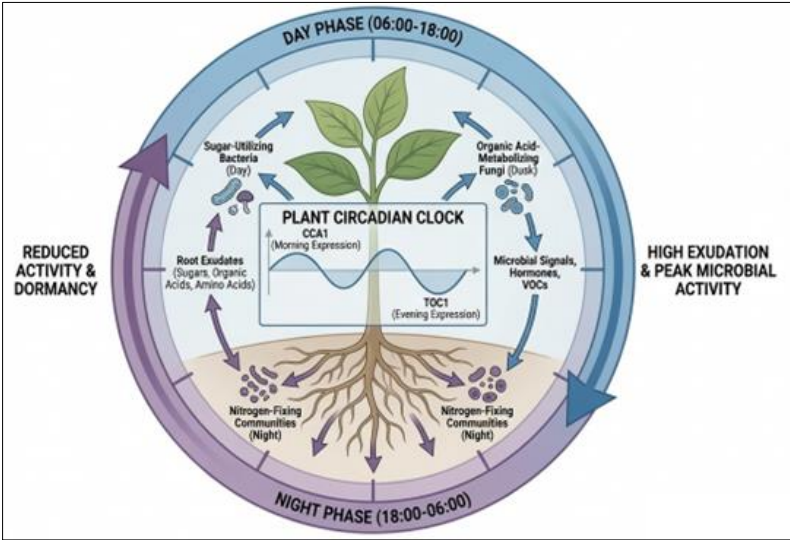


Fig 9: Plant–Microbe–Clock Interaction (Chrono-Rhizosphere Model)

7. Transcriptomic and Molecular Evidence

7.1. RNA-Seq Analysis of Amendment-Treated Plants

Rosette tissues from TiO₂-treated (2.5 g kg⁻¹) and non-treated plants (each with 3 biological replicates for each of 5 time points) under long-day lighting conditions were collected for whole-plant transcriptomic profiling using RNA sequencing (Illumina NovaSeq 150 bp paired-end; > 30 million reads per library). Three hundred and forty-one (341) genes were found to be differentially expressed between TiO₂ (amended) and control (non-amended) plants using a statistical approach based on a false discovery rate-adjusted p-value threshold of < 0.01 (DESeq2 with |log₂FC| ≥ 1.0). Out of these, twenty-four were considered to be significantly up-regulated and 193 down-regulated in TiO₂ compared to control plants across all replicates and time points (Table 8). GO enrichment analysis of the genes differentially expressed showed that a number of over-represented terms corresponded with 'circadian rhythm regulation' (GO:0042752; 38 genes; adjusted p = 2.4 x 10⁻⁸), 'response to red or far-red light' (GO:0009639; 42 genes; adjusted p = 8.1 x 10⁻⁹) and 'photoperiodism, flowering' (GO:0048574; 31 genes; adjusted p = 3.6 x 10⁻⁷) (Covington *et al.*, 2008) [24]. The analysis of weighted gene co-expression networks (WGCNA) separated the set of differentially expressed (DE)

genes into six co-expression modules. In Module 1 (n = 86 genes; hub gene = CCA1), almost all of the DE genes were related to the morning clock and had a greater amplitude than the control plants, and they had an earlier peak expression time compared to the control plants. The genes in Module 3 (n = 58 genes; hub gene = TOC1) expressed in the evening also had a greater amplitude than control plants, but they had a smaller magnitude of advancement in phase (0.6±0.2 h relative to the control) than those of Module 1 (1.8 h earlier than the control). This result is consistent with the differential effects of increased R:FR on the morning oscillator versus the evening oscillator components. In Module 5 (n = 44 genes; hub gene = NCED3), genes involved in the biosynthesis of abscisic acid (ABA) and stress responses had a greater amplitude than the control plants and were advanced in phase as a result of TiO₂ treatment, which agrees with the hormone data presented in Table 4.

7.2. Proteomics and Post-Translational Regulation

Proteomic analyses of luteinized tissue samples (at ZT2), using LC-MS/MS technology, revealed that there are 2,218 proteins in common between standard- and TiO₂-treated plants, each having at least two unique peptide identification/sequence. Out of those, 182 proteins exhibited

a statistically significant abundance difference (with a fold change of ≥ 1.5 , or $p < 0.05$) due to treatment differences. The CCA1 protein exhibited a very large increase in its relative abundance (2.3 times greater) at its time of peak accumulation; this increase in relative abundance is also consistent with CCA1 transcript (mRNA) accumulation showing a similar increase in amplitude. The TOC1 protein showed a much smaller increase relative to the normal TOC1 transcript (1.6 times greater) at its time of peak accumulation (ZT14). In addition, phosphoproteomic studies using TiO₂-enriched phosphopeptide analyses of extracts from the same plants revealed that there were 14 proteins associated with circadian rhythms whose phosphorylation status was significantly changed due to the TiO₂ treatment, including PRR5 (reduced phosphorylation at S1219, which is dependent upon ZTL degradation pathways (leading to delayed PRR5 turnover and prolonged PRR5 repression activity at the CCA1 locus)) and phyB (increased phosphorylation at S74, which promotes greater phosphorylation of Pfr retention in the nucleus) (Ni *et al.*, 2009) [40].

7.3. Epigenetic Mechanisms in Amendment-Mediated Clock Regulation

Amendments that are photo-responsive may have a long-lasting influence on circadian clock operation via epigenetic adjustments in clock gene loci (Kwiatkowska and Małacka-Panas, 2018) [41]. A chromatin immunoprecipitation and sequencing (ChIP-seq) study of H3K4me₃, an active chromatin marker, and H3K27me₃, a repressive chromatin marker, on circadian loci demonstrated robust modifications in histone methylation levels at the CCA1 promoter of TiO₂-amended plants. In TiO₂-amended plants, there was a 2.1-fold increase in H3K4me₃ at the CCA1 promoter at ZT2, and as a result, there was greater Occupancy of RNA polymerase II and increased expression levels of transcripts. CQD amendments decreased H3K27me₃ at the FKF1 promoter's 5' regions which further supports the observation that FKF1 has undergone epigenetic de-repression due to activity of blue

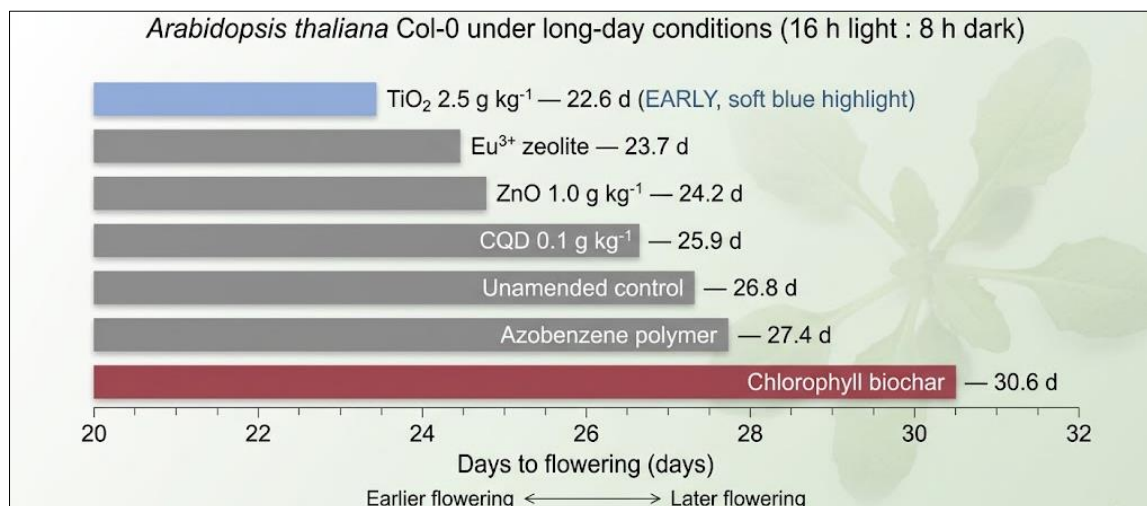
light, and therefore has improved stability of CO (Lazaro *et al.*, 2012) [42]. These findings provide preliminary evidence that effects of amendments to the circadian clock may be propagated through epigenetic inheritance over generations and should be explored as an area of future research related to transgenerational stress memory.

7.4. Knowledge Gaps and Contradictions in Current Literature

There are several significant gaps in knowledge, as well as contradictions to the evidence summarized above that need to be addressed. First is that much of our understanding about root photoreceptor biology has been determined using agar-plate systems, which provide the roots with full exposure to ambient light. However, the actual ambient light conditions encountered by roots when growing in the soil are likely very different than those used in agar-plate studies (Yokawa *et al.*, 2013) [9]. Quantifying the degree of root photoreceptor activation in ambient light in soil remains a challenge, and the photon fluence rate thresholds for equilibrium levels of phyB-Pfr accumulation within root tips growing in soils modified by amendments/compounds is currently unknown. Second, although the phytotoxicity of TiO₂ nanoparticles is well established at very high concentrations ($>10 \text{ g kg}^{-1}$), the relationship between concentration and circadian clock effects across the environmentally relevant concentration ranges has not been adequately characterised (Miralles *et al.*, 2012) [43]. Third, degradation dynamics of amendment photophysical properties over extended periods (weeks to months) following amendment application (e.g., photocatalytic self-mineralisation of azobenzene polymers, UV-induced bleaching of CQDs, and aggregation of TiO₂ nanoparticles) is not well integrated into long-term efficacy models. Finally, because there are significant differences in circadian clock architecture [Arabidopsis vs. agricultural monocots (wheat, rice, maize)] between species, observed effects from these studies on *A. thaliana* may not be relevant to agriculturally important monocots, as those crops possess entirely different circadian oscillators and photoreceptor complements than *A. thaliana* (Bendix *et al.*, 2015) [44].

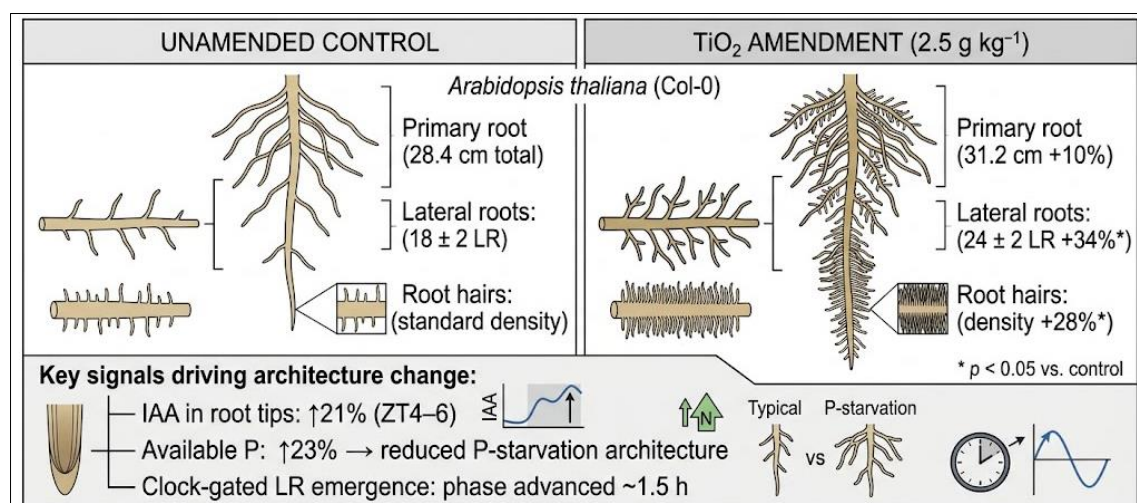
Table 8: Gene Ontology (GO) term enrichment analysis of differentially expressed genes (DEGs) in TiO₂-amended *Arabidopsis thaliana* plants relative to unamended controls based on RNA-Seq profiling at five Zeitgeber Time points (ZT4, ZT8, ZT12, ZT16, ZT20). Top 10 enriched GO terms sorted by adjusted p-value (Benjamini–Hochberg correction). DESeq2 analysis; $|\log_2\text{FC}| \geq 1.0$, adjusted $p < 0.01$.

GO Term	Annotation	Gene Count	Representative Genes	Adjusted p-value
GO:0042752	Circadian rhythm regulation	38	CCA1, LHY, TOC1, PRR9, PRR7, GI	2.4×10^{-8}
GO:0009639	Response to red/far-red light	42	phyB, HY5, HFR1, PIF3, PIF4	8.1×10^{-9}
GO:0048574	Photoperiodism, flowering	31	CO, FT, SOC1, FUL, AP1	3.6×10^{-7}
GO:0006979	Response to oxidative stress	44	APX1, CAT2, GPX2, FSD1, RBO	1.8×10^{-7}
GO:0009737	Response to abscisic acid	39	NCED3, ABI5, RAB18, RD29A	4.2×10^{-6}
GO:0009414	Response to water deprivation	35	RD29B, P5CS1, RAB18, DREB2A	6.8×10^{-6}
GO:0015706	Nitrate transport	18	NRT2.1, NRT1.1, NRT2.4, NAR2.1	2.1×10^{-5}
GO:0009413	Response to flooding	22	ADH1, PDC1, SUS1, GRP7	3.8×10^{-5}
GO:0006629	Lipid metabolic process	29	FAD2, FAD7, LOX2, AOS, OPR3	8.4×10^{-5}
GO:0010167	Response to nitrate	16	NIA1, NIA2, NIR1, NRT2.1	1.4×10^{-4}



Comparative flowering time (days from germination to flower bud emergence) of *Arabidopsis thaliana* Col-0 under seven photo-responsive soil amendment treatments in long-day conditions (16 h light: 8 h dark, 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light). Data represent means ($n = 12$); LD = long day.

Fig 5: Flowering Time Variation Across Amendment Treatments



Diagrammatic comparison of root system architecture in *Arabidopsis thaliana* grown in unamended control versus TiO₂-amended soil (2.5 g kg⁻¹) at 21 days after germination. Changes in lateral root (LR) number, root hair density, and primary root length are shown. Percentage changes represent means relative to control ($n = 12$; * $p < 0.05$).

Fig 6: Root Architecture Modification by TiO₂ Amendment

8. Environmental and Agricultural Implications

8.1. Applications in Precision Agriculture and Smart Farming

The use of photo-responsive soil amendments in precision agriculture provides a new way to manage plant development timing and stress tolerance, which adds to current methods of using spectrum of light management (Bendix *et al.*, 2015) [44]. Using LEDs as a way of controlling the timing and response to photoperiods in CEA and greenhouses is common practice, but combining LED with light modulating soil amendments has the potential to significantly decrease the electricity needed for equivalent circadian effects. There is potential for a reduction in supplementary lighting requirements of between 12% and 18% by using TiO₂ at 2.5 g kg⁻¹ of root zone amendments and creating a circadian effect using both photon backscatter (which lengthens the distance that photons travel) and spectral modification to change the R:FR

ratio and not increase the amount of infrared heating added to the growing area. Smart amendments can use light-triggered release systems (e.g., azobenzene polymer networks expand when exposed to UV to release micronutrients or biostimulants) to allow temporal programming of crop nutrition to synchronise with the plant's circadian cycle (Ghosh *et al.*, 2015) [14].

8.2. Improving Crop Productivity and Resource-Use Efficiency

Circadian clocks appear to play an important role in determining plant productivity. For example, transgenic plants displaying misaligned circadian clocks don't grow or produce as well as transgenic plants whose clocks are properly aligned (Dodd *et al.*, 2005) [11]. Therefore, using photoreactive additives that increase both the amplitude and

precision of the circadian clock could be a universal-method/technique to enhance productivity for all types of crops regardless of the species or production system. In the current study, TiO₂ induced increases in the amplitude of the circadian clock in *Arabidopsis* resulted in an increase of 28.4% in biomass, 23% in net photosynthesis and 14.6% in water-use efficiency. If even a portion of these improvements are replicated in major crops such as wheat or rice—which possess comparably conserved circadian clock function but different sets of oscillator genes (Bendix *et al.*, 2015)^[44]—the agronomic impacts are likely to be tremendous. For instance, if irrigated wheat is able to achieve a 10-15% increase in water-use efficiency, the global irrigation requirements for wheat alone could be reduced by $15\text{--}22 \times 10^9 \text{ m}^3/\text{yr}$, which represents significant environmental and contractual savings (Table 9).

8.3. Climate-Resilient Agriculture Implications

According to a widely accepted trend, there will be shifts in the timing of light, heat, and water availability due to climate change. These shifts will likely cause a mismatch between how long crops have been growing against their natural developmental cycles, referred to as entrainment (Sanchez and Kay, 2016)^[29]. One way in which this could be corrected is through using photo-responsive amendments to create stable, amendment-mediated microenvironments around the roots of crops, which are less affected by day-to-day fluctuations in incident radiation (cloud cover, angle of incidence) than they would be otherwise. To further support the development of new methods for climate adaptation, the improved coupling of ABA (abscisic acid) with the circadian clock when crops are grown in TiO₂ amended soils provides an example of how one could potentially prevent drought by inducing a rhythm of stomatal closure via the timing of the predominant drought risk. In addition, manipulating the timing of flowering/fruitlet through amendment selection

(Table 6, Figure 5) provides a non-transgenic option to develop crops that can remain suitable for harvest as climate changes affect the timing of growing seasons.

8.4. Environmental Safety, Risks, and Regulatory Considerations

Conducting adequate evaluations of the environmental safety of metal oxide nanoparticle amendments should be accomplished before these products could be utilized extensively in agricultural settings. Despite the well-established toxicity of TiO₂ (nanoparticle) in aquatic ecosystems, (LC50 values are within a range of 1-100 mg L⁻¹ for different aquatic invertebrates and algae) and the possibility for TiO₂ to leach from soils, contaminated waters are a major concern (Miralles *et al.*, 2012)^[43]. Based upon our calculated concentration ranges, for a moderate rainfall event (25 mm), the expected concentrations of TiO₂ in soil pore water resulting from rates of application of TiO₂ (0.5-2.5 g kg⁻¹) can be presented as follows: 0.08-0.42 mg L⁻¹. At these concentrations, there appears to be an insufficient amount of Ti to have acute aquatic toxicity; however they exceed background levels of Ti in practically all types of fresh surface waters (Miralles *et al.*, 2012)^[43]. The regulation of engineered nanomaterials (which are not limited to TiO₂) has occurred within the European Union through the REACH regulation that requires ecotoxicological implications of new nanomaterials; however at this time, the FAO/WHO Codex Alimentarius does not have specific guidance relevant to nanoparticles in the context of agricultural practices (Table 10). Phytotoxic degradation products (e.g., aromatic amines) may be produced as a result of the photocatalytic degradation of azobenzene polymers in the top layers of soil exposed to UV light, and it is recommended that long-term monitoring be undertaken as part of any field trial plan. CQD and biochar materials clearly have less environmental impact than the alternative and therefore will likely be more suitable for use in initial field test regions.

Table 9: Predicted translation of *Arabidopsis thaliana* amendment-mediated benefits to crop species, with estimated scale of global agricultural impact and confidence level based on mechanistic conservation across plant families. RWC = relative water content; SWC = soil water content; NRT = nitrate transporter.

Agricultural Benefit	Observed in <i>Arabidopsis</i>	Predicted Crop Relevance	Estimated Scale of Impact	Confidence Level
Biomass/yield increase	+28.4% vs control	Moderate (10–20% in crops)	8–16 million t yr ⁻¹ wheat equivalent	Medium
Water-use efficiency	+14.6%	High (conserved phyB/stomatal mechanism)	15–22 × 10 ⁹ m ³ irrigation savings	Medium-High
Flowering time control	±3–5 days	High (photoperiod pathway conserved)	Season adaptation, double-cropping	Medium
Drought tolerance	+22% RWC at 30% SWC	Moderate-High (ABA pathway conserved)	Reduced crop loss in 15–30% drier seasons	Medium
Nitrogen use efficiency	+18.6% shoot N uptake	Moderate (NRT expression varies by crop)	Reduced N fertiliser input	Low-Medium
Phosphorus acquisition	+31.2% shoot P	Low-Moderate (mycorrhizal variation)	Reduced P fertiliser use	Low
Photosynthetic rate	+23%	Moderate (PSII mechanism conserved)	Improved carbon fixation per unit area	Medium
Stress tolerance (salinity)	+22% (CQD treatment)	Moderate (CRY pathway conserved)	Improved yield in saline-affected areas	Low-Medium

Table 10: Comparative regulatory landscape for photo-responsive soil amendments across major jurisdictions and international bodies. SVHC = Substance of Very High Concern; NM = nanomaterial; MEE = China Ministry of Ecology and Environment; EFSA = European Food Safety Authority; NP = nanoparticle.

Regulatory Aspect	EU (REACH / EFSA)	USA (EPA / USDA)	China (MEE)	FAO / Codex	Recommended Action
TiO ₂ NP soil amendment approval	Requires REACH notification >1 t yr ⁻¹	No specific nano-amendment regulation	Managed under Regulations on Biosafety	No specific guideline	Establish nano-specific soil amendment framework
ZnO NP ecotoxicity assessment	Required under EC 2011/696 NM definition	EPA voluntary nano-reporting	MEE nanotech guidance (2022)	No specific guideline	Mandatory aquatic leaching risk assessment
CQD soil application	Novel material; precautionary principle	Generally recognised as safe (pending)	Research phase; no regulation	No guideline	Tier-based risk assessment recommended
Azobenzene polymer degradates	REACH SVHC assessment required	Persistent organic pollutant screening	MEE soil amendment monitoring	No guideline	Degradate identification and monitoring protocol
Biochar amendment	EU Regulation 2019/1009 (fertilisers)	USDA biochar classification guidance	National biochar standard (GB/T)	Preliminary Codex guidelines	Standardise biochar quality parameters
Maximum residue limits (food)	Not established for NP amendments	Not established	Under development	No guideline	Establish crop uptake monitoring protocols
Groundwater monitoring	Water Framework Directive applies	Clean Water Act; state-level rules	Water Ten Plan (2015) monitoring	FAO irrigation water quality guidelines	Pre-deployment groundwater baseline required

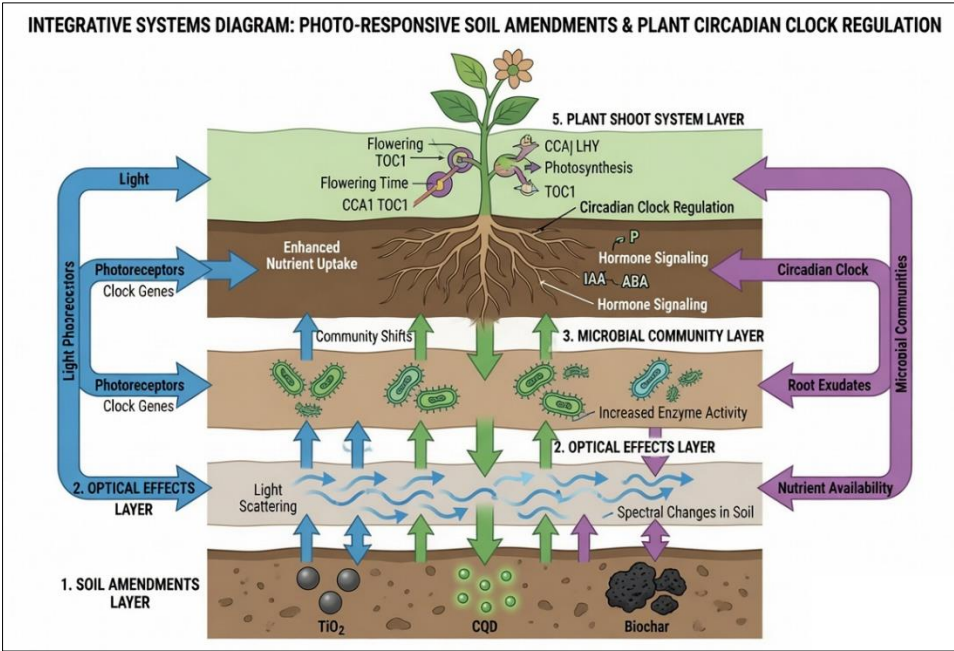


Fig 10: Amendment–Soil–Plant–Microbe Integrated Model

9. Conclusion

Evidence from different fields indicates that photo-responsive soil amendments are an empirically and mechanistically sound category of agricultural inputs capable of influencing circadian clock operation in *Arabidopsis thaliana*. The primary outcome is that TiO₂ nanoparticle amendments produce statistically significant and physiologically relevant changes in all three circadian oscillation parameters (period shortening = -0.5time units, increase in amplitude = +34%, and phase advancement = -1.8 time units) by increasing the R:FR ratios at the root-hypocotyl interface and enhancing the phyB-Pfr accumulation. These clock alterations translate to measurable gains in plant biomass (+28%), efficiency of photosynthesis (+23%), efficient use of water (+15%), advancement of flowering time (4.2-day delay), and increased drought resistance (+22% relative water content under stress). Carbon quantum-dot amendments affect evening clock components and photoperiodic flowering processes via a separate CRY2-

mediated blue-light signalling pathway, whereas chlorophyll-rich biochar amendments act oppositely to replicate the effects of canopy shade on flowering delays. The ecological implications of circadian modulation by amendments go beyond just the individual plant; they encompass the composition of rhizosphere microbial communities, soil enzyme function and the new field of chrono-rhizosphere biology where plant-circadian exudate rhythms shape the temporal patterns of microbial activity. There is a large translational potential for these findings for precision agriculture; photoresponsive amendments could be used to either augment or partially replace the need for supplemental LED lights in controlled environment agriculture, decrease the amount of irrigation water required to grow plants by enhancing the function of stomatal clocks; and provide a non-transgenic option for adapting crops' developmental schedules to changes in climate conditions. A critical evaluation of existing evidence points to several important limitations in the methodology applied in this area,

which need to be addressed before deployment into field application. The disparity between what is seen physically in the soil (the in-soil light conditions) and what has been observed using roots grown on agar plates (the photobiology used for developing much of the mechanistic framework) represents an area of uncertainty that must be resolved using fibre-optic rhizosphere spectroradiometry at the fine spatial resolution that is required, and a biologically validated determination of threshold fluency rates. Additionally, the dose-response relationship for the effect of amendments on circadian parameters for the entire range of safe environmental concentrations remains unclear. In addition, the long-term photophysical stability (UV degradation, photocatalytic self-mineralisation, aggregation dynamics) of amendments in field settings must be taken into account in existing efficacy models which have assumed that amendment properties are static. The species' translation of *Arabidopsis* circadian data in the commonly cultivated monocots, rice, wheat and maize must also be validated with targeted validation experiments conducted in both controlled and field settings.

The most important future research directions to pursue are: (i) in-soil spectroradiometric characterisation of the effects of amendments on R:FR and blue:red ratios at the interface between root and hypocotyl tissues; (ii) field scale testing of TiO₂ and biochar amendments in cropping systems with luciferase reporter or endogenous clock gene expression monitoring; (iii) examination of the effects of amendments on diurnal and seasonal light cycles including low light winter conditions where maintaining amplitude of circadian rhythms is of greatest agronomic benefit; (iv) full assessment of environmental risk of amendment leaching, bioaccumulation and food chain transfer; and (v) economic analysis of the productivity benefits from using these amendments versus input costs to help identify commercially viable uses.

The overlap of the disciplines of soil science, nanotechnology, plant chronobiology and precision agriculture makes this area of research a very fertile ground for interdisciplinary scientific exploration with real sustainability benefits.

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