

## Dynamic Simulation of Leaf Area Index in Flue-Cured Tobacco Based on Nitrogen Effect: Post-print

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### Abstract

To clarify the dynamic characteristics of leaf area index (LAI) in flue-cured tobacco populations under different nitrogen application levels and its relationship with active accumulated temperature, this study used ‘Yuyan 12’, ‘Qinyan 96’, and ‘Yunyan 87’ as experimental materials, with four nitrogen application levels: N0 (0 kg · hm<sup>-2</sup>), N1 (30 kg · hm<sup>-2</sup>), N2 (60 kg · hm<sup>-2</sup>), and N3 (90 kg · hm<sup>-2</sup>). The leaf area index of flue-cured tobacco populations and its dynamic characteristics under different accumulated temperatures were measured and analyzed. A normalized accumulated temperature model was established using Curve Expert 1.40 software for simulation and the limit value method for screening, providing a theoretical basis for improving the photosynthetic structure of flue-cured tobacco populations. The results showed that: 1) The leaf area index of flue-cured tobacco populations exhibited a unimodal curve variation with skewness <0 with active accumulated temperature after transplanting, and showed an increasing trend with increasing nitrogen application levels. Under the same nitrogen application level, the peak leaf area index of flue-cured tobacco populations followed the order: ‘Qinyan 96’ > ‘Yunyan 87’ > ‘Yuyan 12’. 2) The rational function model  $y=(a+bx)/(1+cx+dx^2)$  demonstrated good simulation performance and biological significance, effectively simulating the variation of relative leaf area index in flue-cured tobacco populations with relative active accumulated temperature, with a coefficient of determination of 0.980 7\*\*. The model was validated using experimental data from 2015, showing simulation accuracy (k) greater than 0.958, precision (R2) greater than 0.95, and root mean square error (RMSE) less than 6.04%. 3) Model parameters exhibited significant differences among certain varieties and nitrogen application levels, with variety and nitrogen amount primarily regulating the entire simulation model through adjusting model parameters b, c, and d. 4) The change rate curve of relative leaf area index in flue-cured tobacco

populations exhibited an 'N' -shaped pattern, reflecting the actual variation trend of leaf area index in flue-cured tobacco populations. 5) Nitrogen application amount had a regulatory effect on model secondary parameters. With increasing nitrogen application, the average leaf area index and maximum leaf area index of flue-cured tobacco populations showed increasing trends, which could serve as important reference indicators for nitrogen regulation of leaf area index in flue-cured tobacco populations. The establishment of this model can provide theoretical basis and decision support for dynamic monitoring of flue-cured tobacco population development and improvement of leaf photosynthetic characteristics.

## Full Text

### Dynamic Simulation of Leaf Area Index of Tobacco Based on Nitrogen Effect

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## Abstract

To clarify the dynamic characteristics of leaf area index (LAI) in flue-cured tobacco populations under different nitrogen application levels and its relationship with active accumulated temperature, this study examined three tobacco cultivars ('Yuyan 12', 'Qinyan 96', and 'Yunyan 87') under four nitrogen levels: N0 (0 kg · hm<sup>-2</sup>), N1 (30 kg · hm<sup>-2</sup>), N2 (60 kg · hm<sup>-2</sup>), and N3 (90 kg · hm<sup>-2</sup>). LAI and its dynamic characteristics were measured and analyzed at different accumulated temperatures. Using Curve Expert 1.40 software and limit value method screening, a normalized accumulated temperature model was established to provide a theoretical basis for improving the photosynthetic structure of tobacco populations. The results showed that: (1) Tobacco population LAI exhibited a unimodal curve with skewness < 0 following transplanting, increasing with nitrogen level. At the same nitrogen level, peak LAI followed the order 'Qinyan 96' > 'Yunyan 87' > 'Yuyan 12'. (2) The rational function model  $y = (a + bx)/(1 + cx + dx^2)$  demonstrated excellent simulation performance and biological significance, effectively modeling the relationship between relative LAI and relative active accumulated temperature with a determination coefficient of 0.9807\*\*. Model validation using 2015 data showed simulation accuracy (k) > 0.958, precision (R<sup>2</sup>) > 0.95, and root mean square error (RMSE) < 6.04% for all cases. (3) Model parameters showed significant differences among certain cultivars and nitrogen levels, with cultivar and nitrogen level primarily regulating the entire model through parameters b, c, and d. (4) The change rate

curve of relative LAI displayed an “N-shaped” pattern, reflecting the actual dynamic trend of tobacco population LAI. (5) Nitrogen application rate regulated secondary parameters, with both mean LAI and maximum LAI increasing with nitrogen level, serving as important reference indicators for nitrogen regulation of tobacco population LAI. This model provides theoretical basis and decision support for dynamic monitoring of tobacco population development and improvement of leaf photosynthetic characteristics.

## Keywords

flue-cured tobacco; leaf area index; active accumulated temperature; nitrogen application level; rational function model

## Introduction

Leaves are the primary organs for photosynthesis, transpiration, and organic matter synthesis in crops, and their functional traits directly reflect plant genetic characteristics and resource utilization efficiency. Photosynthesis forms the foundation for crop yield and quality formation, and leaf area, as an important functional trait, significantly influences crop population photosynthesis and the entire growth process. Leaf area index (LAI) is a crucial parameter for quantifying population structure, reflecting the capacity of crops to utilize solar radiation energy for photosynthesis. Within a certain range, crop yield increases with LAI; however, when LAI exceeds a threshold, excessive leaf overlap causes canopy closure, reduces light intensity, decreases photosynthetic rate, and consequently lowers crop yield. Therefore, simulating LAI dynamics is essential for obtaining crop growth status information and is critical for quantitatively analyzing the entire crop growth process. An optimal LAI also provides a rational basis for developing high-yield crop population structures. To understand crop population LAI patterns, quantitative research using model equations to simulate LAI dynamics has become a research hotspot.

Flue-cured tobacco (*Nicotiana tabacum* L.) is an economic crop cultivated for leaf harvest, with yield formation representing a process of leaf area expansion and dry matter accumulation. Quantitative analysis of tobacco LAI dynamics plays an important role in monitoring growth status, disease and pest surveillance, and field management. Previous LAI research in tobacco has been limited, often focusing on canopy spectra integration. Liu et al. established a stepwise regression model combining hyperspectral parameters with tobacco LAI, demonstrating good simulation performance. Zhang et al. investigated the precision of different hyperspectral models for LAI detection, concluding that principal component analysis and neural network methods could improve inversion accuracy, with validation model determination coefficients reaching 0.938 and 0.889, respectively. Temperature is a major ecological factor affecting leaf area expansion, yet few studies have reported on the relationship between tobacco LAI and ecological factors. Zhang et al. established a tobacco leaf dry matter produc-

tion model based on integrated light-temperature indices, providing a scientific approach for tobacco yield prediction. As a special crop, flue-cured tobacco is harvested after maturity through multiple picking sessions. Many studies have only considered pre-maturity changes without addressing post-maturity LAI changes during harvest, representing a significant limitation. Therefore, establishing a direct dynamic model between temperature and LAI throughout the entire growth period could provide theoretical basis and decision support for understanding tobacco population development dynamics. To further investigate tobacco LAI dynamic patterns, and considering that a given tobacco cultivar has relatively stable accumulated temperature requirements throughout its growth cycle, this study used active accumulated temperature instead of days after transplanting as the measurement scale. Referencing LAI dynamic simulation models from other crops and employing a relative normalization method, we established an accumulated temperature model relating relative active accumulated temperature to relative LAI under different nitrogen levels. Through model analysis, we examined dynamic characteristics and parameter changes of tobacco population LAI under different nitrogen regulation levels and explored the feasibility of using LAI dynamic simulation as an effective means for monitoring tobacco field growth status. These findings hold significant importance for improving photosynthetic efficiency and dynamic monitoring of tobacco growth processes.

## Materials and Methods

### Experimental Site Description

The experiments were conducted in 2015 and 2016 at the tobacco base unit experimental field in Xiaojie Township, Luoning County, Luoyang City, Henan Province (111°38 N, 34°26 E, altitude 567 m). During the tobacco field growth period (May–September), the mean temperature was 24.36°C with 437.15 mm rainfall and 835.2 h sunshine in 2015, and 23.59°C with 415.56 mm rainfall and 867.3 h sunshine in 2016. The experimental soil was yellow loam with wheat (*Triticum aestivum* L.) as the previous crop. The site featured flat terrain and abundant sunlight. Basic soil physicochemical properties for 2015 and 2016 are presented in Table 1 .

Both years employed a split-plot design with nitrogen application rate and cultivar as factors. The main plot consisted of four nitrogen levels: N0 (0 kg · hm<sup>-2</sup>), N1 (30 kg · hm<sup>-2</sup>), N2 (60 kg · hm<sup>-2</sup>), and N3 (90 kg · hm<sup>-2</sup>). The sub-plot comprised three cultivars: ‘Yuyan 12’ (YU12), ‘Qinyan 96’ (QIN96), and ‘Yunyan 87’ (YUN87). Twelve treatment plots were established, each 120 m<sup>2</sup> with 200 plants at a row spacing of 1.20 m × 0.55 m. For each nitrogen treatment, 70% of nitrogen was applied as base fertilizer at the bottom of ridges during ridging, with the remaining 30% applied at the rosette stage. Fertilizers included tobacco-specific compound fertilizer, sesame cake fertilizer, potassium sulfate, potassium nitrate, and calcium superphosphate at an N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O ratio of 1:1.5:3. Transplanting dates were May 13, 2015, and May 4, 2016. Topping,

removal of bottom leaves, and sequential harvest of mature leaves are essential agronomic practices in tobacco production and represent significant sources of LAI measurement error. While unavoidable, these operational errors were minimized to improve analytical accuracy by maintaining consistent topping and bottom leaf removal standards, timely harvesting of only mature leaves, and uniform field management across all plots according to local high-quality tobacco production technical specifications.

### Tobacco Population LAI Measurement

At the rosette stage, ten uniformly growing plants per plot were tagged and measured from that date. For each treatment, three plants were selected during each measurement to record the length and width of all expanded leaves (all immature expanded leaves attached to the plant). Measurements were taken every 3–5 days during early rapid growth and every 8–10 days during later stages, with additional measurements before topping and bottom leaf removal. LAI was calculated using Formula (1):

$$LAI = \frac{k \times \rho \times \sum_{i=1}^a \sum_{j=1}^n (L_{ij} \times W_{ij})}{a}$$

where  $k$  is the tobacco leaf area correction coefficient (0.6345),  $\rho$  is planting density (1.65 plants  $\cdot$  m<sup>-2</sup> in this experiment),  $a$  is the number of measured plants, and  $L_{ij}$  and  $W_{ij}$  represent the maximum length and width of the  $j$ th leaf on the  $i$ th plant, respectively.

Daily meteorological data during the tobacco field growth period were recorded by an automatic weather station 600 m from the experimental field. Active accumulated temperature (TEM) after transplanting was calculated following the method of Yan et al.

### Data Normalization

LAI and TEM data from 2015 and 2016 were processed using Microsoft Excel 2016 and normalized to obtain relative leaf area index (RLAI) and relative active accumulated temperature (RTEM) using the following formulas:

$$RLAI_i = \frac{LAI_i}{LAI_{max}} \quad (0 \leq RLAI_i \leq 1) \quad (2)$$

$$RTEM_i = \frac{TEM_i}{TEM_{max}} \quad (0 \leq RTEM_i \leq 1) \quad (3)$$

where  $RLAI_i$  and  $RTEM_i$  are the relative leaf area index and relative active accumulated temperature at different growth stages,  $LAI_i$  and  $TEM_i$  are the measured LAI and TEM at different stages, and  $LAI_{max}$  and  $TEM_{max}$  are the maximum LAI and TEM values from transplanting to maturity.

## Model Establishment and Validation

Curve Expert 1.40 software was used to dynamically simulate RLAI and RTEM data from 2016, generating dynamic simulation equations. The limit value method was employed to screen for biologically meaningful RLAI dynamic models. The 2015 dataset was used to test model accuracy and precision. Since tobacco growth has relatively stable accumulated temperature requirements, any RTEM value could be substituted into the equation to obtain corresponding RLAI simulated values. Measured LAI values were normalized to obtain RLAI observed values for model validation. With simulated RLAI as the x-axis and observed RLAI as the y-axis, linear equations ( $y = kx$ ) were established. The proximity of coefficient  $k$  to 1 reflected simulation accuracy, while the coefficient of determination ( $R^2$ ) indicated precision. SPSS 21.0 was used for one-way ANOVA with LSD multiple comparisons, and Microsoft Excel 2016 was used for figure and table preparation.

## Results

### Dynamic Characteristics of Tobacco Population LAI

The LAI curves across different nitrogen levels and cultivars showed consistent trends, all exhibiting unimodal patterns with skewness  $< 0$  (Fig. 1 [Figure 1: see original paper]). LAI initially increased with post-transplant accumulated temperature, peaking at approximately 1200°C, then gradually declining. Peak LAI at the same nitrogen level followed the order 'QIN96'  $>$  'YUN87'  $>$  'YU12', with 'YU12' and 'YUN87' showing flatter peaks than 'QIN96'. At lower TEM, LAI differences among nitrogen levels were small but increased with accumulated temperature. The steepness of the post-peak decline reflected the amount of mature leaves harvested and thus the maturation rate, with maturation rates ranking as 'QIN96'  $>$  'YUN87'  $>$  'YU12'.

### Dynamic Changes of Relative LAI (RLAI)

Normalization of LAI and post-transplant TEM eliminated dimensional differences and facilitated comparison. RLAI trends across different nitrogen levels were consistent with measured LAI, showing unimodal curves with skewness  $< 0$  (Fig. 2 [Figure 2: see original paper]). 'YU12' exhibited the flattest peak, followed by 'YUN87' and 'QIN96'. Flatter peaks indicated prolonged maintenance of higher LAI levels and greater photosynthetic production potential but slower maturation rates. Differences among nitrogen levels were minimal at low RTEM but became apparent at higher RTEM, with N0 showing the most pronounced RLAI decline.

### Dynamic Simulation of RLAI

Eight well-fitting models were developed using Curve Expert 1.40 and screened using the limit value method. As  $x \rightarrow \infty$ ,  $y$  should approach 0. However, Models

2, 3, 6, 7, and 8 approached infinity or constants, lacking biological meaning. Models 4 and 5 were undefined at  $x = 0$ . Therefore, these models were rejected in favor of Model 1 for simulating RLAI dynamics.

Model 1 is a rational function:  $y = (a + bx)/(1 + cx + dx^2)$ . At  $x = 0$ ,  $y = a = 0.0201$ , representing RLAI at transplanting. As  $x \rightarrow \infty$ ,  $y = 0$ , corresponding to  $LAI = 0$  after complete leaf harvest. The single peak represents maximum RLAI. The model effectively simulated RLAI changes with parameters  $a = 0.0201$ ,  $b = 0.2785$ ,  $c = -3.3122$ ,  $d = 3.3019$ , standard deviation = 0.0425, and  $R^2 = 0.9807^{**}$  (highly significant) (Fig. 3 [Figure 3: see original paper]).

### Key Parameter Analysis of RLAI Dynamic Model

Normalized dynamic simulation equations were established for each 2016 treatment (Table 3), all with  $R^2 \geq 0.9220$ , confirming the model's applicability across cultivars and nitrogen levels. Significance analysis revealed differential effects of cultivar and nitrogen on parameters. For cultivars, parameter  $b$  showed no significant differences, while parameter  $a$  was not significantly different between 'YUN87' and 'YU12', and parameters  $c$  and  $d$  were not different between 'QIN96' and 'YUN87'. All other parameters differed significantly ( $P < 0.05$ ). For nitrogen levels, parameter  $d$  showed no significant differences, parameters  $b$  and  $c$  were not different among N0, N1, and N2, and parameter  $a$  was not different between N0-N1 and N1-N2. All other parameters differed significantly ( $P < 0.05$ ). Although parameter  $a$  varied among cultivars and nitrogen levels, its values were close to 0, indicating minimal impact. Cultivar and nitrogen primarily regulated the model through parameters  $b$ ,  $c$ , and  $d$ .

### Analysis of RLAI Dynamic Model Change Rate

The first derivative of the RLAI model  $y = (a + bx)/(1 + cx + dx^2)$  yields the change rate equation:  $y' = (b - ac - 2adx - bdx^2)/(1 + cx + dx^2)^2$ . Substituting RTEM produced change rate curves showing "N-shaped" patterns across all treatments (Fig. 4 [Figure 4: see original paper]). Values  $> 0$  indicated increasing RLAI with initially accelerating then decelerating rates, peaking at RTEM 0.4 (rapid growth stage). When the change rate equaled 0 at RTEM 0.5, tobacco entered the maturity stage. Values  $< 0$  indicated decreasing RLAI with initially accelerating then decelerating decline rates. Under N0, maximum increase and decrease rates were higher in 'QIN96' and 'YU12' than other nitrogen levels, with minimal differences among nitrogen levels. In 'YUN87', N1 showed the highest rates with more dispersed curves among nitrogen levels, possibly reflecting differential nitrogen responsiveness.

### Validation of RLAI Dynamic Optimization Model

The 2015 dataset validated the RLAI model (Fig. 5 [Figure 5: see original paper]). Simulated and observed values were closely matched across three cultivars, with simulation accuracy ( $k$ ) of 0.981, 0.988, 0.975, and 0.958 (all near

1), precision ( $R^2$ ) of 0.9785, 0.9748, 0.9607, and 0.9672 (all  $> 0.95$ ), and RMSE of 4.52%, 4.71%, 6.04%, and 5.91% (all relatively small). The model demonstrated high precision and accuracy, effectively reflecting tobacco population LAI dynamics and meeting prediction requirements.

### Maximum LAI (LAI<sub>max</sub>) and Nitrogen Application

Maximum LAI indicates population assimilation capacity and corresponds to the peak in dynamic models. LAI<sub>max</sub> showed significant linear positive correlation with nitrogen rate (0-90 kg · hm<sup>-2</sup>), with higher nitrogen producing greater LAI<sub>max</sub> (Fig. 6 [Figure 6: see original paper]). Regression equations were  $y = 0.0134x + 3.3895$  ( $R^2 = 0.9724$ ),  $y = 0.0114x + 3.4053$  ( $R^2 = 0.9798$ ), and  $y = 0.0095x + 2.8847$  ( $R^2 = 0.9563^*$ ) for ‘QIN96’, ‘YU12’, and ‘YUN87’, respectively. ‘YUN87’ had significantly lower LAI<sub>max</sub> than ‘QIN96’ and ‘YU12’, while the latter two showed no significant difference.

### Mean LAI (MLAI) and Nitrogen Application

MLAI reflects population dry matter accumulation throughout the growth period. Integrating RLAI yields mean relative LAI (MRLAI), and  $MLAI = MRLAI \times LAI_{max}$ , calculated using formulas (4) and (5):

$$MRLAI = \frac{ad - bc}{d} \arctan\left(\frac{2dT_2 + c}{\sqrt{4d - c^2}}\right) - \arctan\left(\frac{2dT_1 + c}{\sqrt{4d - c^2}}\right)$$

$$MLAI = MRLAI \times LAI_{max}$$

where  $T_1$  and  $T_2$  are relative accumulated temperatures. Setting  $T_1 = 0$  and  $T_2 = 1$  yields MRLAI for the entire growth period. MLAI increased significantly with nitrogen level, with regression equations of  $y = 0.0073x + 1.7571$  ( $R^2 = 0.993^{**}$ ),  $y = 0.0058x + 1.4446$  ( $R^2 = 0.994$ ), and  $y = 0.0052x + 1.5067$  ( $R^2 = 0.999$ ) for ‘YU12’, ‘YUN87’, and ‘QIN96’, respectively. Increased nitrogen significantly affected MLAI, enhancing production potential. ‘YU12’ showed significantly higher MLAI than ‘YUN87’ and ‘QIN96’, while the latter two showed no significant difference.

## Discussion

As a temperature-sensitive crop, tobacco responds strongly to thermal conditions. Studies show both high and low temperatures affect normal growth and photosynthesis. This study investigated the LAI-accumulated temperature relationship because tobacco has relatively stable accumulated temperature requirements. LAI increased then decreased with accumulated temperature in a unimodal pattern, consistent with studies on maize and cotton. Establishing mathematical models for crop growth dynamics using modern information technology is crucial for obtaining crop development status. Models enable LAI

prediction, scientific growth monitoring, and timely large-scale crop assessment, providing decision support for yield estimation and cultivation management.

This study examined tobacco LAI dynamics under nitrogen and accumulated temperature regulation, establishing a dynamic model. The rational function  $y = (a + bx)/(1 + cx + dx^2)$  effectively simulated RLAI changes with RTEM, with few parameters and simple calculation requiring only TEM to retrieve LAI at any time, providing theoretical support for high-quality tobacco production.

Nitrogen is the primary factor limiting tobacco growth and quality among all essential nutrients, directly determining plant nutrition status and leaf yield/quality. Studies show single leaf mass increases with nitrogen level, and since leaf mass largely depends on leaf area, nitrogen affects population leaf area. Appropriate nitrogen promotes coordinated growth, increases net photosynthetic rate, and facilitates quality formation; excessive nitrogen causes dark green leaves and delayed maturation, postponing LAI<sub>max</sub> and reducing maturation rate; insufficient nitrogen slows growth, lowering LAI<sub>max</sub>. This study confirmed LAI<sub>max</sub> increased with nitrogen while maturation rate decreased, most notably in 'YUN87', consistent with previous findings. Proper nitrogen application can improve leaf photosynthetic performance and provide material basis for chemical component transformation.

The RLAI model regulates LAI simulation through parameters. Parameter *a* (near zero) showed minimal regulation by cultivar or nitrogen, which primarily controlled the model through parameters *b*, *c*, and *d*. The model also calculated secondary characteristic parameters like LAI<sub>max</sub> and MLAI for comprehensive growth evaluation. MLAI is a secondary parameter reflecting whole-growth-period dry matter production, with increased MLAI raising average crop growth rate. This study found MLAI increased with nitrogen level, with effects more pronounced in later stages. Both MLAI and LAI<sub>max</sub> increased with nitrogen, with cultivar differences: 'YU12' had highest MLAI, while 'YUN87' had lowest LAI<sub>max</sub>, possibly due to cultivar characteristics or nitrogen use efficiency differences. Optimal nitrogen levels are required to balance population production level and maximum potential.

The LAI change rate accurately reflects growth speed at different stages. Studies in spring maize and different planting dates showed "N-shaped" LAI change rate curves. This study found tobacco RLAI change rate followed an "N-shaped" pattern with accumulated temperature, derived from model differentiation with practical production significance. The maximum rate occurred at approximately 900°C accumulated temperature (about 65 days after transplanting) during the rapid growth stage. When the rate reached zero at approximately 1100°C (about 80 days after transplanting), tobacco entered maturity and RLAI began negative change. Nitrogen effects were minor on 'YU12' and 'QIN96' but substantial on 'YUN87', possibly related to differential nitrogen responsiveness. The rate curve enables timely monitoring of RLAI changes and LAI dynamics.

This study only explored RLAI-RTEM relationships under different nitrogen lev-

els with preliminary validation. Tobacco leaf growth is also affected by water, fertilizer, planting density, temperature, radiation, and light. Comprehensive factor regulation models require further research. However, this study incorporated mature leaf harvest into the research scope, which previous studies had not addressed.

## Conclusion

Tobacco population LAI showed unimodal curves with accumulated temperature, increasing with nitrogen level, with ‘QIN96’ showing the highest peak and fastest maturation rate. RLAI also exhibited unimodal curves with RTEM, with ‘YU12’ showing the flattest peak and N0 level showing the fastest decline after harvest. Using RTEM as the independent variable and normalization methods, the dynamic model  $y = (0.0201 + 0.2785x)/(1 - 3.3122x + 3.3019x^2)$  effectively predicted tobacco LAI dynamics with  $R^2 = 0.9807$ , accuracy (k) > 0.958, precision ( $R^2$ ) > 0.95, and RMSE < 6.04%. Cultivar and nitrogen affected model parameters, primarily through b, c, and d. Secondary parameters like MLAI, LAImax, and RLAI change rate responded to nitrogen, with MLAI and LAImax increasing with nitrogen level (‘QIN96’ had highest LAImax; ‘YU12’ had highest MLAI). The RLAI change rate curve showed “N-shaped” patterns with cultivar and nitrogen level differences. Nitrogen application rate thus serves as an important indicator for improving tobacco population photosynthetic structure and coordinating yield and quality.

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*Note: Figure translations are in progress. See original paper for figures.*

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